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(54) Title: METHODS FOR DETECTING AND IDENTIFYING A GRAM POSITIVE BACTERIA IN A SAMPLE

(57) Abstract: The present invention provides fragments of a sodA gene from gram positive bacteria, methods of using these fragments as probes to detect and identify microorganisms in a sample and kits containing suitable reagents to perform the method.

# METHODS FOR DETECTING AND IDENTIFYING A GRAM POSITIVE BACTERIA IN A SAMPLE

## FIELD OF THE INVENTION

The invention relates to probes and methods of detecting and identifying microorganisms, particularly gram-positive bacteria, in test samples.

## BACKGROUND OF THE INVENTION

Enterococci, although not highly virulent microorganisms, have emerged worldwide in the last decade as one of the leading causes of nosocomial bacteremia, surgical wound infections, and urinary tract infections (9, 10, 13, 24). This evolution is mainly due to the appearance of multiresistant strains of enterococci that can be resistant to most antibiotics used for the treatment (ampicillin, aminoglycosides, and glycopeptides). Most human enterococcal infections ( 90%) are caused by *Enterococcus faecalis and Enterococcus faecium,* however, the incidence of other species, such as *Enterococcus casseliflavus* and *Enterococcus gallinarum,* could be underestimated because of bacterial mis-identification. In clinical laboratories, accurate identification of enterococcal species is required to carry out a proper epidemiologic surveillance and may help in the management of infected patients in case of relapse. This is usually done by testing tolerance to bile esculine and tellurite, growth in 6.5% NaCl broth, specific carbohydrate utilization (2, 6), by characterizing bacterial motility and pigment production (1), and by using commercial biochemical test systems, such as the API-20 STREP or rapid ID 32 Strep. However, these phenotypic methods are often not reliable and the automated systems, such as the

Vitek and MicroScan systems, do not properly identify enterococci other than *E. faecalis and E. faecium* in absence of additional tests (11). Consequently, several genotypic methods based on the analysis of PCR products derived from selected target DNA have been developed for species identification of enterococci (3, 14, 22). This includes the determination of the 16S rDNA sequence (18), a strategy which is now greatly facilitated by the use of universal 16S PCR primers associated with the development of simplified, partially automated, and cost effective sequencing technologies. However, the interpretation of these data may be complicated by the fact that divergent 16S rDNA sequences may exist within a single organism (23) or, alternatively, that closely related species may have identical 16S rDNA sequences (8), as recently shown in the genera *Enterococcus* for E. casseliflavus and *E. gallinarum* (18). To solve this problem, it is possible to use alternative monocopy target sequences which exhibit a higher divergence than that of the 16S rDNA. The *sodA* gene of the gram positive cocci which encodes the manganese-dependent superoxide dismutase fulfills these criteria and we recently reported that sequencing of the *sodA* PCR product with the use of a single pair of degenerate primers constitutes a valuable approach to the genotypic identification of the 29 streptococcal species (20). In this work, the same universal primers (19) were used to construct a *sodA* database of 19 enterococcal species including *E. casseliflavus* and *E. gallinarum*.

## SUMMARY OF THE INVENTION

The present invention provides polynucleotides capable of hybridizing specifically to nucleic acids of the *sodA* gene from gram positive bacteria, methods of

using these polynucleotides as probes to detect and identify microorganisms in a sample, and kits containing suitable reagents to perform the methods.

In particular, the invention provides methods for accurate identification of the species of a gram positive bacteria in a sample comprising providing a sample suspected of containing said gram positive bacteria; hybridizing a specific probe for a *sodA* gene or a fragment thereof to nucleic acids from said microorganism; and detecting the presence or absence of hybridization. In preferred embodiments, said microorganism is selected from the group consisting of *Enterococci, Abiotrophia, Streptococci* and *Staphylococci*. Probes and methods of the invention may preferably relate to the detection of a *Enterococci* selected from the group consisting of *E. avium, E. casseliflavus, E. cecorum, E. columbae, E. dispar, E. durans, E. faecalis, E. faecium, E. flavescens, E. gallinarum, E. hirae, E. malodoratus, E. mundtii, E. pseudoavium, E. raffinosus, E. saccharolyticus, E. seriolicida, E. solitarius,* and *E. sulfureus*. In other preferred embodiments, probes and methods of the invention may preferably relate to the detection of a *Staphyloccus* selected from the group consisting of *S.arlettae, S. auricularis, S. capitis* subspecies *capitis, S. capitis* subspecies *ureolyticus, S. caprae, S. carnosus* subspecies *carnosus, S. carnosus* subspecies *utilis, S. chromogenes, S cohnii* subspecies *cohnii, S. cohnii* subspecies *urealyticum, S. condimenti, S. delphini, S epidermidis, S. equorum, S felis, S. gallinarum, S. haemolyticus, S. hominis* subspecies *hominis, S. hominis* subspecies *novobiosepticus, S. hyicus, S. intermedius, S. kloosii, S. lentus, S. lugdunensis, S. luntae, S. muscae, S. pasterui, S. piscifermentans, S. pulvereri, S. saccharolyticus, S. saprophyticus* subspecies *bovis, S. saprophyticus* subspecies *saprophyticus, S schleiferi* subspecies

*coagulans, S. schleiferi* subspecies *schleiferi, S. sciuri* subspecies *carnaticus, S. sciuri* subspecies *sciuri, S. simulans, S vitulinus, S. warneri,* and *S. xylosus.*

The present invention also provides polynucleotides specific for a *sodA* gene for use in hybridization assays for the detection of the presence or absence of gram-positive bacteria. In preferred embodiments, the invention provides polynucleotides specific for the $sodA_{int}$ region of the sodA gene, including the polynucleotide probes of SEQ ID NOS 1 to 94, or the complements thereto, or fragments or derivatives thereof. Further provided are DNA chips comprising at least one polynucleotide of the invention. Provided are also polynucleotides or fragments thereof specifically hybridizing to an Enterococcus microorganism, wherein SEQ ID NO:1 is specific for *E. avium,* SEQ ID NO:2 is specific for *E. casseliflavus,* SEQ ID NO:3 is specific for *E. cecorum,* SEQ ID NO:4 is specific for *E. columbae,* SEQ ID NO:5 is specific for *E. dispar,* SEQ ID NO:6 is specific for *E. durans,*SEQ ID NO:7 is specific for *E. faecalis,* SEQ ID NO:8 is specific for *E. faecium,* SEQ ID NO:9 is specific for *E. flavescens,* SEQ ID NO:10 is specific for *E. gallinarum,* SEQ ID NO:11 is specific for *E. hirae,* SEQ ID NO:12 is specific for *E. malodoratus,* SEQ ID NO:13 is specific for *E. mundtii,* SEQ ID NO:14 is specific for *E. pseudoavium,* SEQ ID NO:17 is specific for *E. raffinosus,* SEQ ID NO:15 is specific for *E. saccharolyticus,* SEQ ID NO:18 is specific for *E. seriolicida,* SEQ ID NO:16 is specific for *E. solitarius,* and SEQ ID NO:19 is specific for *E. sulfureus.* Provided are polynucleotides or fragments thereof specifically hybridizing to a microorganism of the genus *Enterococci*, wherein SEQ ID NOS:21-36 are specific for species in the *Enterococci*; polynucleotides or fragments thereof specifically hybridizing to a microorganism of the genus *Lactococcus garvieae*, wherein said polynucleotide is SEQ ID NO: 20; polynucleotides or fragments thereof

specifically hybridizing to a microorganism of the genus *Streptococcus*, wherein SEQ ID NOS:37-50 are specific for species in the *Streptococci*; polynucleotides or fragments thereof specifically hybridizing to a microorganism of the genus *Abiotrophia*, wherein SEQ ID NOS:51-53 are specific for species in the *Abiotrophia*; and polynucleotides or fragments thereof specifically hybridizing to a microorganism of the genus *Staphlococcus*, wherein SEQ ID NOS:54-93 are specific for species in the *Staphlococcus*.

In particularly preferred embodiments, the invention encompasses methods for the identification of a gram positive bacterial species selected from the group consisting of *Streptococci*, *Staphylococci*, *Abiotrophia* and *Enterococci* comprising (a) selecting a polynucleotide of about 425 to 445 bp comprised between two conserved domains of SOD gene said polynucleotide having flanking regions consisting in two oligonucleotidic sequences and being specific for the genus or the species to be detected; (b) hybridizing the DNA of the sample with the polynucleotide; (c) washing the hybridized sample; and (d) visualizing the reaction of hybridization with an electric or electronic or calorimetric system.

In preferred embodiments, the methods of the invention comprise hybridizing a probe specific to the $sodA_{int}$ fragment of the *sodA* gene.

In further preferred embodiments, methods of the invention may comprise amplifying said *sodA* gene from the microorganism prior to said hybridizing.

Also provided are isolated or purified polynucleotides comprising, consisting essentially of, of consisting of the nucleotide sequence of SEQ ID NOS 1 to 94, and the complements thereof, or fragments thereof. Said polynucleotides may comprise at

least 12, 18, 20, 30, 50, 75, 100, 200, 300, 400, 450 or 500 contiguous nucleotides, to the extent the length of said span in consistent with the length of the SEQ ID, of a nucleotide sequence selected from the group consisting of SEQ ID NOS 1 to 94. Envisioned also are polynucleotides having at least 90% and preferably at least 95%, 97%, 98%, 99%, 99.8% or 99.9% sequence identity with a polynucleotide of SEQ ID NOS 1 to 94, or a fragment thereof. Percent identity can be determined for example electronically, e.g., by using the MegAlign.TM. program (DNASTAR, Inc., Madison Wis.), or default parameters for nucleic acid comparisons in the "gap" program from Genetics Computer Group, Madison Wis. (algorithm of Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)). The invention also relates to a kit for the detection of a gram positive bacteria present in a sample containing at least a polynucleotide of SEQ ID NOS 1 to 94. Also encompassed is a 400 bp polynucleotide sequence obtained after amplification of a DNA template from a sample by using a pair of primers SEQ ID NOS:95 and 96, wherein said pair of primers is specific for the SOD gene of a gram positive bacteria. In further embodiments, the polynucleotide is a polynucleotide of about 429bp and specific for a *Staphylococi* species; a polynucleotide of about 435 and specific for *Streptococci* species; a polynucleotide of about 438 bp and specific for *Enterococci* species; or a polynucleotide of about 438 to 441 bp and specific for Abiotrophia species.

The present invention is directed to polynucleotide probes specific for nucleotide sequences of the sodA gene for use in diagnostic methods, preferably hybridization-based assays, for the detection of specific strains of gram positive bacteria in a biological sample. Detection of specific sodA polynucleotides in a eukaryote, particularly a mammal, and especially a human, provides a diagnostic

method for diagnosis of disease, staging of disease or response of an infectious organism to drugs. In some embodiments, one or multiple probes, or panels of probes comprising probes specific for one or more species of gram positive bacteria, particularly species of *Enterococci, Abiotrophia, Streptococci* and *Staphylococci,* may be used in assays to detect the presence or absence of said bacteria in samples suspected to be contained in a biological sample.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Phylogenetic unrooted tree showing the relationships among the $sodA_{int}$ fragments from various enterococcal type strains. The tree was established from an analysis of the sequences listed in Table 1 by using the neighbor-joining method. The $sodA_{int}$ sequences of *L. lactis, L. garvieae, S. bovis, S. pyogenes* type strains were included in this work. The value on each branch is the estimated confidence limit (expressed as a percentage) for the position of the branch as determined by bootstrap analysis. Only the bootstrap values superior to 95%, which were considered as significant, are indicated. The scale bar (NJ distance) represents 10% differences in nucleotide sequences.

Figure 2: An identity matrix based on pairwise comparisons of $sodA_{int}$ fragments of enterococcal type strains. The main characteristics of each of the strains listed in Fig. 2 are listed in Table 1.

## DETAILED DESCRIPTION OF THE INVENTION

Unless defined otherwise, all technical and scientific terms and any acronyms used herein have the same meanings as commonly understood by one of ordinary skill

in the art in the field of the invention. Although any methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred methods, devices, and materials are described herein.

All patents and publications mentioned herein are incorporated herein by reference to the extent allowed by law for the purpose of describing and disclosing the proteins, enzymes, vectors, host cells, and methodologies reported therein that might be used with the present invention. However, nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Fragments from sodA genes from a number of Enterococcus species are shown in SEQ ID NOS:1-19 and 21-36, from Lactococcus garvieae is shown in SEQ ID NO:20, from a number of Streptococcus species are shown in SEQ ID NOS:37-50, from a number of Abiotrophia species are shown in SEQ ID NOS:51-53, from a number of Staphlococcus species are shown in SEQ ID NOS:54-93 and from Macrococcus caseolyticus is shown in SEQ ID NO:94.

Microbial specimens for use in this invention can be obtained from any source suspected of harbouring bacteria. The samples are generally dispersed in a measured amount of buffer, though dispersal may be optimal if lysis is immediately possible. This dispersal buffer generally provides a biologically compatible solution. Samples may be frozen or used directly after obtaining.

Prior to analysis, samples suspected of containing bacteria are preferably subjected to a lysing solution to release cellular nucleic acids. Dispersal of the sample prior to lysis is optional. Lysing buffers are known in the art (Ausubel et al (eds), Current Protocols in Molecular Biology, John Wiley and Sons, Inc., 2000). Generally,

these buffers are between pH 7.0 and 8.0, and contain both chelating agents and surfactants. Typically, a lysing solution is a buffered detergent solution having a divalent metal chelator or a buffered chaotrophic salt solution containing a detergent (such as SDS), a reducing agent and a divalent metal chelator (EDTA). The use of enzymes such as N-acetyl-muramidase (lysozyme) or proteases (such as Protease K) will facilitate lysis and offer high quality results.

The sample may be directly immobilized to a support or further processed to extract nucleic acids prior to immobilization. Released or extracted bacterial nucleic acid (including target nucleic acid) are fixed to a solid support, such as cellulose, nylon, nitrocellulose, diazobenzyloxymethyl cellulose, and the like. The immobilized nucleic acid can then be subjected to hybridization conditions.

Alternatively, samples may be collected and dispersed in a lysing solution that also functions as a hybridization solution, such as 3M guanidinium thiocyanate (GuSCN), 50 mM Tris (pH 7.6), 10 mM EDTA, 0.1% sodium dodecylsulfate (SDS), and 1% mercaptoethanol (Maniatis, T. et al. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, N.Y., 1982).

Alternatively, the nucleic acid probes may be immobilized onto solid phase microchips according to methods known in the art and subsequently hybridization with sample nucleic acids can be identified with a microchip reader. This and other solid phase microchip methods are disclosed in Ausebel et al (supra). Detection systems comprising a high-density array library of probes immobilized on a substrate are also known, described in PCT Application No. WO 97 02357 (Affymetrix Inc.), U.S. Patent No. 5,202,231 (Drmanac), U.S. Patent No. 6,228,575 (Affymetrix). Essentially any desired number of probes can be used in said array or microchip; for example at

least 1, 2, 10, 100, 1000 or more nucleic acid probes may be immobilized. Arrays or microchips may also include sets of nucleic acid probes comprising at least 1, 2, 5, 10, 20 or 50 nucleotide sequences of SEQ ID NOS 1 to 94, or fragments, complements and/or derivatives thereof.

Various degrees of stringency of hybridization can be employed. As the conditions for hybridization become more stringent, there must be a greater degree of complementarity between the probe and the target for duplex formation to occur. Stringency conditions for hybridization is a term of art which refers to the conditions of temperature and buffer concentration which permit hybridization of a particular nucleic acid to a second nucleic acid in which the first nucleic acid may be perfectly complementary to the second, or the first and second may share some degree of complementarity which is less than perfect. For example, certain high stringency conditions can be used which distinguish perfectly complementary nucleic acids from those of less complementarity. "High stringency conditions" and "moderate stringency conditions" for nucleic acid hybridizations are explained on pages 2.10.1-2.10.16 (see particularly 2.10.8-11) and pages 6.3.1-6 in Current Protocols in Molecular Biology (Ausubel, F. M. et al., eds., Vol. 1, containing supplements up through Supplement 29, 1995). Hybridization techniques are also generally described in Hames, et al. (eds.), "Nucleic Acid Hybridization, A Practical Approach", IRL Press, New York, 1985. The degree of stringency can be controlled by temperature, ionic strength, pH and the presence of a partially denaturing solvent such as formamide. For example, the stringency of hybridization is conveniently varied by changing the polarity of the reactant solution through manipulation of the concentration of formamide within the range of 0% to 50%. Stringency also depends on factors such as the length of the

nucleic acid sequence, base composition, percent mismatch between hybridizing sequences and the frequency of occurrence of subsets of that sequence within other non-identical sequences. Thus, high or moderate stringency conditions can be determined empirically.

By varying hybridization conditions from a level of stringency at which no hybridization occurs to a level at which hybridization is first observed, conditions which will allow a given sequence to hybridize with the most similar sequences in the sample can be determined. Exemplary conditions are described in Ausubel et al in Current Protocols in Molecular Biology (supra), including descriptions regarding how to determine washing conditions at page 2.10.11. Washing is the step in which conditions are usually set so as to determine a minimum level of complementarity of the hybrids and to eliminate non-hybridizing labelled probe as well as background and non-specific weak interactions. Generally, starting from the lowest temperature at which only homologous hybridization occurs, each degree C by which the final wash temperature is reduced (holding SSC concentration constant) allows an increase by 1% in the maximum extent of mismatching among the sequences that hybridize. Generally, doubling the concentration of SSC results in an increase in $T_m$ of about 17 C. Using these guidelines, the washing temperature can be determined empirically for high, moderate or low stringency, depending on the level of mismatch sought. For example, conditions may be determined such that hybridization occurs only if there is at least 90% and preferably at least 95%, 97%, 98%, 99%, 99.8% or 99.9% identity between the sequences.

In practicing the present invention, amplification of either the nucleic acid probe or a sodA gene from the microorganism sample may be performed prior to the

hybridization. Examples of amplification techniques include Strand Displacement Amplification (i.e., SDA, also described in Walker G. T. et al., 1992, Nucleic Acids Res., 20:1691-1696), the Polymerase Chain Reaction (i.e., PCR), Reverse Transcription Polymerase Chain Reaction (i.e., RT-PCR), Nucleic Acid Sequence-Based Amplification (i.e., NASBA), Self-Sustained Sequence Replication (i.e., 3SR), and the Ligase Chain Reaction (i.e., LCR). (see, e.g. Innis et al., PCR Protocols, a Guide to Methods and Applications, eds., Academic Press (1990)).

The primers used to amplify the sample nucleic acids are oligonucleotides of defined sequence selected to hybridize selectively with particular portions of the sodA gene, in particular those that amplify the sodA internal fragment ($sodA_{int}$). A primer or primer pair may be coupled to a detectable moiety.

Polynucleotides including probes and primers and primer pairs may comprise any suitable detectable moiety. Examples of detectable moieties or labels include fluorescein, which is a standard label used in nucleic acid sequencing systems using laser light as a detection system. Other detectable labels can also be employed, including enzymes, cofactors, enzyme substrates, other fluorophores, chemiluminescent molecules, radio-labels ($^{32}P$, $^{35}S$, $^{3}H$, $^{125}I$), chemical couplers such as biotin which can be detected with streptavidin-linked enzymes, and epitope tags such as digoxigenin detected using antibodies. Other examples are described in French Patent No. FR-7810975 or by Urdea M. S. et al., 1991, Nucleic Acids Symp. Ser., 24:197-200.or Sanchez-Pescador R., 1988, J. Clin. Microbiol., 26(10) :1934-1938. Probes can also be prepared as "capture probes", and are for this purpose immobilized on a substrate in order to capture the target nucleic acid contained in a biological sample. The captured target nucleic acid is subsequently detected with a

second probe, which recognizes a sequence of the target nucleic acid that is different from the sequence recognized by the capture probe.

Polynucleotides may be synthesized by any of several well known methods, including automated solid-phase chemical synthesis using cyano-ethylphosphoramidite precursors. Barone, A. D. et al., Nucleic Acids Research 12, 4051-4060 (1984). Methods of preparing probes and determing the quality of probe compositions is generally well known (see for example U.S. Patent No. 5,994,059). Probes or primers also can be prepared by cleavage of the polynucleotides by restriction enzymes, as described in Sambrook et al. in 1989.

The present invention concerns methods for identification of species by a method which comprises providing a sample suspected of containing a gram positive bacteria, hybridizing a specific probe for a sodA gene or fragment thereof to nucleic acids from the microorganism, and detecting the presence or absence of hybridization. More specifically, the present invention concerns a method for the identification of a gram positive bacterial species selected from the group consisting of *Streptococci, Staphlococci, Abiotrophia,* and *Enterococci,* wherein the method has the steps of selecting a polynucleotide of 400 to 500 bp comprised between two conserved domains of SOD gene said polynucleotide having flanking regions consisting in two oligonucleotidic sequences and being specific for the genus or the species to be detected; hybridizing the DNA of the sample with the polynucleotide; washing the hybridized sample; visualizing the reaction of hybridization with an electric or electronic or calorimetric system. A polynucleotide of about 425 to 445 bp is particularly preferred.

The present invention also includes diagnostic kits for performing the analysis. These kits can be used to facilitate detection and identification of specific bacterial species in a clinical laboratories. Such kits would include instruction cards and vials containing the various solutions necessary to conduct a nucleic acid hybridization assay. These solutions would include lysing solutions, hybridization solutions, combination lysing and hybridization solutions, and wash solutions. The kits would also include labeled probes. The UP9A probe could be either unlabeled or labeled depending on the assay format. Standard references for comparison of results would also be necessary to provide an easy estimate of bacterial numbers in a given solution. Depending upon the label used additional components may be needed for the kit, e.g. enzyme labels require substrates.

Having generally described this invention, a further understanding can be obtained by reference to certain specific examples which are provided herein for purposes of illustration only, and are not intended to be limiting unless otherwise specified.

## EXAMPLES

The main characteristics of the bacterial strains used in this study, including the type strains, are listed in Table 1 and 2. Rapid extraction of bacterial genomic DNA was carried out by using the InstaGene$^{Tm}$ Matrix (Bio-Rad, Hercules, CA) on cells collected from 2 ml of an overnight culture. The *sodA* degenerate primers *dl* (5'-CCITAYICITAYGAYGCIYTIGARCC-3') (SEQ ID NO:95) and *d2* (5'-ARRTARTAIGCRTGYTCCCAIACRTC-3') (SEQ ID NO:96) were used to amplify an internal fragment designated sodA$_{int}$ representing approximately 85% of their *sodA* genes. PCRs were performed on a Gene Amp System 9600 instrument (Perkin Elmer

Cetus, Roissy, France) in a final volume of 50 µl containing 250 ng of DNA as template, 0.5 µM of each primer, 200 µM of each dNTP, and 1 U of AmpliTaq Gold DNA polymerase (Perkin Elmer) in a 1X amplification buffer (10 mM Tris-HCI [pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$). The PCR mixtures were denatured (3 min at 95 C), then subjected to 30 cycles of amplification (60 s of annealing at 37 C, 60 s of elongation at 72 C, and 30 s of denaturation at 95 C), and 72 C for 7 min for the last elongation cycle. A single DNA fragment corresponding to the expected 480-bp amplification product, *sodA*$_{int}$, was observed in all cases following agarose gel electrophoresis and ethidium bromide staining (data not shown). PCR products were purified on a S-400 Sephadex column (Pharmacia, Uppsala, Sweden) and directly sequenced on both strands with the oligos *d1* and *d2* by using the ABI-PRISM® big dye terminator sequencing kit on a Genetic ABI-PRISM® 310 Sequencer Analyzer (Perkin Elmer). The cycle sequencing protocol was optimized as follows: the sequencing mixtures were subjected to 40 cycles of amplification consisting of 10 s of denaturation at 96 C, 5 s of annealing at 40 C, and 4 min of elongation at 60 C.

The nucleotide sequences of the *sodA*$_{int}$ fragments from the type strains of *E. avium, E. casseliflavus, E. cecorum, E. columbae, E. dispar, E. durans, E. faecalis, E. faecium, E. flavescens, E. gallinarum, E. hirae, E. malodoratus, E. mundtii, E. pseudoavium, E. raffinosus, E. saccharolyticus, E. seriolicida, E. solitarius, E. sulfureus*, and *Lactococcus garvieae* were determined (Table 1). We assumed that the PCR products sequenced were actual *sodA*$_{int}$ fragments since the corresponding deduced polypeptides all contained the amino acids characteristic of the manganese-dependent superoxide dismutase (16, 17) at the expected positions (data not shown). Multiple alignment of these *sodA*$_{int}$ DNA sequences plus those from *L. garvieae* (Table

1), *Lactococcus lactis* (19), *Streptococcus bovis* (20) and *Streptococcus pyogenes* (20) was carried out by the Clustal X program (12) and an unrooted phylogenetic tree was constructed by the neighbor-joining (NJ) method (21). The sequence of the degenerate oligonucleotides *d1* and *d2* and alignment gaps were not taken into consideration for calculations. The reliability of the tree nodes was evaluated by calculating the percentage of 1,000 bootstrap resamplings that support each topological element. Only the nodes having a bootstrap value greater than 95% are indicated in Fig. 1 since this critical value could be used to define the monophyly of a clade of related organisms (7). This analysis revealed that, as expected, the members of the genus *Enterococcus*, with the exception of *E. seriolicida* were clustered within a clade supported by 99.5% of the bootstrap replicates. The $sodA_{int}$ sequences of *E. seriolicida* and of *L. garvieae* were almost identical (99.5% of sequence identity) and were clustered with that of *L. lactis* within a clade supported by 96.3% of the bootstrap confidence (Fig 2 and Fig. 1). These results are consistent with the redesignation of *E. seriolicida* as *L. garvieae* (4). The phylogenetic tree representing the enterococcal $sodA_{int}$ sequences (Fig. 1) has the same topology as the NJ tree constructed from the analysis of their 16S rDNA sequences (18). It is worth noting that the $sodA_{int}$ sequences of *E. casseliflavus and E. gallinarum* type strains displayed 16.9% of sequence divergence, a value similar to the 19.7% of sequence divergence observed between the *ddl* genes encoding the D-Ala-D-Ala ligases in these species (5). These results do not support the suggestion that *E. casseliflavus* and *E. gallinarum* comprise a single species (18). By contrast, the fact that the 16S rDNA (18), the *ddl* (15), the *vanC* (3), and the $sodA_{int}$ (Fig. 2) genes of *E. casseliflavus* and *E. flavescens* type strains were almost identical (99.9, 99.5%, 96%,

and 98% of sequence identity, respectively) suggest that they should be associated in a single species.

The phylogenetic tree showed in Fig. 1 revealed the presence of two major clusters within the enterococcal species which we have designated the *faecium* group (*E. faecium*, *E. durans*, *E. hirae*, and *E. mundtii*) and the *avium* group *(E. avium, E. malodoratus, E. pseudoavium, and E. raffinosus).* Within each group, the 16S rDNA sequences exhibited more than 99% of sequence identity (18) whereas the highest percentage of similarity found between two $sodA_{int}$ sequences was 87.9% (Fig. 2). These results confirm that the gene *sodA* constitutes a more discriminative target sequence than the 16S RNA to differentiate closely related bacterial species.

Fifteen enterococcal isolates were identified by using conventional microbiological tests, ID 32 Strep, and the $sodA_{int}$ systems (Table 2). In all cases, the $sodA_{int}$ sequences of the isolates displayed less than 1.5% of divergence with that of the corresponding type strain. For ten strains (NEM1616, NEM1617, NEM1621, NEM1623, NEM1624, NEM1625, NEM1626, NEM1627, NEM1628, and NEM1630), the two methods gave the same results. Four isolates (NEM1618, NEM1620, NEM1622, AND NEM1629) were identified at the species level with *the* $sodA_{int}$ system but not with the conventional microbiological tests and the ID 32 Strep system. The remaining isolate NEM1619 was identified with the ID 32 Strep system as *E. hirae* but was identified with the $sodA_{int}$ system as *E. durans* (Table 2). The reliability of the molecular identification of NEM1164 was based on the fact that its $sodA_{int}$ fragment displays 99.5% and 85% of sequence identity with those of the type strains of *E. durans and E. hirae,* respectively.

In conclusion, we have determined the $sodA_{int}$ sequences of the type strains of *E. avium, E. casseliflavus/E. flavescens, E. cecorum, E. columbae, E. dispar, E. durans, E. faecalis, E. faecium, E. gallinarum, E. hirae, E. malodoratus, E mundtii, E. pseudoavium, E. raffinosus, E. saccharolyticus, E. seriolicida, E. solitarius, and E. sulfureus* and demonstrated the usefulness of this database for the species identification of enterococcal isolates. The identification method presented in this study is not accessible to routine clinical microbiology laboratories but it may become the gold-standard technique in reference and large research hospital laboratories for epidemiologic purposes and/or to identify problematic strains.

Other polynucleotide sequences specific for species of *Staphlococci*, *Streptococci* and *Abiotrophia* have also been identified by using the same method. These sequences correspond to SEQ ID NOS:54-59, SEQ ID NOS:37-58 and SEQ ID NOS:51-53, respectively. Corresponding strains and culture collection designations are set forth in the sequence listing.

TABLE 1. Enterococcal type strains used in this study

| Strain[a] | Other designation[b] | $sodA_{int}$ accession n° |
|---|---|---|
| *E. avium* CIP 103019 T | ATCC 14025 | AJ387906 |
| *E. casseliflavus* CIP 103018 T | ATCC 25788 | AJ387907 |
| *E. cecorum* CIP 103676 T | ATCC 43198 | AJ387908 |
| *E. columbae* CIP 103675 T | ATCC 51263 | AJ387909 |
| *E. dispar* CIP 103646 T | ATCC 51266 | AJ387910 |
| *E. durans* CIP 55.125 T | ATCC 19432 | AJ387911 |
| *E. faecalis* CIP 103015 T | ATCC 19433 | AJ387912 |
| *E. faecium* CIP 103014 T | ATCC 19434 | AJ387913 |
| *E. flavescens* CIP 103525 T | ATCC 49996 | AJ387914 |
| *E. gallinarum* CIP 103013 T | ATCC 49573 | AJ387915 |
| *E. hirae* CIP 53.48 T | ATCC 8043 | AJ387916 |
| *E. malodoratus* CIP 103012 T | ATCC 43197 | AJ387917 |
| *E. mundtii* CIP 103010 T | ATCC 43186 | AJ387918 |
| *E. pseudoavium* CIP 103647 T | ATCC 49372 | AJ387919 |
| *E. saccharolyticus* CIP 103246 T | ATCC 43076 | AJ387920 |
| *E. solitarius* CIP 103330 T | NCTC 12193 | AJ387921 |
| *E. raffinosus* CIP 103329 T | ATCC 49427 | AJ387922 |
| *E. seriolicida* CIP 104369 T | ATCC 49156 | AJ387923 |
| *E. sulfureus* CIP 104373 T | DSM 6905 | AJ387924 |
| *L. garvieae* CIP 102507 T | DSM20684 | AJ387925 |

[a] CIP, Collection de l'Institut Pasteur.

[b] ATCC, American Type Culture Collection; DSM, Deutsche Sammlung Von Mikrooganismen; NCTC, National Collection of Type Cultures.

TABLE 2. Identification of various enterococcal strains by sequencing the *sodA$_{int}$* fragment.

| Strain | Relevant characteristics [a] | Bacterial species [b] | Accession number |
|---|---|---|---|
| NEM1616 | *E. faecalis*; *vanA* | *E. faecalis* (99.5) | AJ387927 |
| NEM1617 | *E. faecalis*; *vanA* | *E. faecalis* (98.6) | AJ387928 |
| NEM1618 | *Enterococcus* sp. | *E. durans* (99.3) | AJ387929 |
| NEM1619 | *E. hirae* | *E. durans* (99.5) | AJ387930 |
| NEM1620 | *Enterococcus* sp. | *E. durans* (99.1) | AJ387931 |
| NEM1621 | *E. hirae* | *E. hirae* (99.8) | AJ387932 |
| NEM1622 | *Enterococcus* sp. | *E. hirae* (99.5) | AJ387933 |
| NEM1623 | *E. casseliflavus* | *E. casseliflavus* (99.1) | AJ387934 |
| NEM1624 | *E. faecium*; *vanB* | *E. faecium* (99.5) | AJ387935 |
| NEM1625 | *E. faecium*; *vanA* | *E. faecium* (100) | AJ387936 |
| NEM1626 | *E. faecium*; *vanB* | *E. faecium* (99.8) | AJ387937 |
| NEM1627 | *E. faecium*; multiply resistant strain | *E. faecium* (99.8) | AJ387938 |
| NEM1628 | *E. faecium*; multiply resistant strain | *E. faecium* (99.8) | AJ387939 |
| NEM1629 | *Enterococcus* sp. | *E. gallinarum* (98.6) | AJ387940 |
| NEM1630 | *E. avium* | *E. avium* (100) | AJ387941 |

[a] Bacterial strains were all clinical isolates from our collection which were identified by using conventional microbiological tests and the ID 32 Strep System (API-bio-Mérieux). Presence of *vanA* (NEM1616, NEM1617, and NEM1625) and *vanB* (NEM1624 and NEM1626) was determined by PCR with specific primers (3).

[b] The species identification was based on the phylogenic position of the *sodA$_{int}$* fragment of the strain studied relative to those of the type strains, as shown in Fig.1. The number in parentheses indicates the percentage of identity of the *sodA$_{int}$* fragment with that of the corresponding type strains.

## REFERENCES

The following references are referred to by number in the specification of the present application. Other references are included in the body of the specification.

1. **Cartwright, C. P., F. Stock, G. A. Fable, and V. J. Gill.** 1995. Comparison of pigment production and motility tests with PCR for reliable identification of intrinsically vancomycin-resistant enterococci. J. Clin. Microbiol. 33:1931-.1933.

2. **Devriese, L. A., B. Pot, K. Kersters, S. Lauwers, and F. Haesebrouck.** 1996. Acidification of methyl-alpha-D-glucopyranoside: a useful test to differentiate *Enterococcus casseliflavus* and *Enterococcus gallinarum* from *Enterococcus faecium* species group and from *Enterococcus faecalis*. J. Clin. Microbiol. 34:2607-2608.

3. **Dutka-Malen, S., S. Evers, and P. Courvalin.** 1995. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J. Clin. Microbiol. 33:1434.

4. **Eldar, A., C. Ghittino, L. Asanta, E. Bozzetta, M. Goria, M. Prearo, and H. Bercovier.** 1996. *Enterococcus seriolicida* is a junior synonym of *Lactococcus garvieae,* a causative agent of septicemia and meningoencephalitis in fish. Curr. Microbiol. 32:85-88.

5. **Evers, S., B. Casadewall, M. Charles, S. Dutka-Malen, M. Galimand, and P. Courvalin.** 1996. Evolution of structure and substrate specificity in D-alanine:D-alanine ligases and related enzymes. J. Mol. Evol. 42:706-712.

6. **Facklam, R. R., and M. D. Collins.** 1989. Identification of *Enterococcus species* isolated from human infections by a conventional test scheme. J. Clin. Microbiol. **27**:731-734.

7. **Felsenstein, J.** 1985. Confidence limits on phylogeny and approach using the boostrap. Evolution **39**:783-791,

8. **Fox, G. E., J. D. Wisotzkey, and P. Jurtshuk, Jr.** 1992. How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. Int. J. Syst. Bacteriol. **42**:166-170.

9. **Hunt, C. P.** 1998. The emergence of enterococci as a cause of nosocomial infection. Br. J. Biomed. Sci. **55**:149-156.

10. **Huycke, M. M., D. F. Sahm, and M. S. Gilmore.** 1998. Multiple-drug resistant enterococci: the nature of the problem and an agenda for the future. Emerg. Infect. Dis. **4**:239-249.

11. **Iwen, P. C., D. M. Kelly, J. Linder, and S. H. Hinrichs.** 1996. Revised approach for identification and detection of ampicillin and vancomycin resistance in *Enterococcus* species by using MicroScan panels. J. Clin. Microbiol. **34**:1779-1783.

12. **Jeanmougin, F., J. D. Thompson, M. Gouy, D. G. Higgins, and T. J. Gibson.** 1998. Multiple sequence alignment with Clustal X. Trends Biochem. Sci. **23**:403-405.

13. **Moellering, R. C., Jr.** 1998. Vancomycin-resistant enterococci. Clin. Infect. Dis. **26**:1196-1199.

14. **Monstein, H. J., M. Quednau, A. Samuelsson, S. Ahrne, B. Isaksson, and J. Jonasson.** 1998. Division of the genus *Enterococcus* into species groups using PCR-based molecular typing methods. Microbiology **144**:1171-1179.

15. **Navarro, F., and P. Courvalin.** 1994. Analysis of genes encoding D-alanine-D-alanine ligase-related enzymes in *Enterococcus casseliflavas* and *Enterococcus flavescens*. Antimicrob. Agents Chemother. **38**:1788-1793.

16. **Parker, M. W., and C. C. F. Balke.** 1988. Crystal structure of manganese superoxide dismutase from *Bacillus stearothermophilus* at 2.4 A resolution, J. Mol. Biol. **199**:649-661.

17. **Parker, M. W., and C. C. F. Blake.** 1988. Iron- and manganese-containing superoxide dismutases can be distinguished by analysis of their primary structures. FEBS Lett. **229**:377-382.

18. **Patel, R., K. E. Piper, M. S. Rouse, J. M. Steckelberg, J. R. Uhl, P. Kohnerg M. K. Hopkins, F. R. Cockerill, 3rd, and B. C. Kline.** 1998. Determination of 16S rRNA sequences of enterococci and application to species identification of nonmotile *Enterococcus gallinarum* isolates. J. Clin. Microbiol. **36**:3399-3407.

19. **Poyart, C., P. Berche, and P. Trieu-Cuot.** 1995. Characterization of superoxide dismutase genes from Gram-positive bacteria by polymerase chain reaction using degenerate primers, FEMS Microbiol. Lett. **131**:41-45.

20. **Poyart, C., G. Quesne, S. Coulon, P. Berche, and P. Trieu-Cuot.** 1998. Identification of streptococci to species level by sequencing the gene encoding the manganese-dependent superoxide dismutase. J. Clin. Microbiol. **36**:41-47.

21. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**:406-425.

22. **Tyrrell, G. J., R. N. Bethune, B. Willey, and D. E. Low.** 1997. Species identification of enterococci via intergenic ribosomal PCR. J. Clin. Microbiol. **35**:1054-1060.

23. **Ueda, K., T. Seki, T. Kudo, T. Yoshida, and M. Kataoka.** 1999. Two distinct mechanisms cause heterogeneity of 16S rRNA. J. Bacteriol. **181**:78-82.

24. **Woodford, N.** 1998. Glycopeptide-resistant enterococci: a decade of experience. J. Med. Microbiol. **47**:849-862.

CLAIMS

1. A method for accurate identification of the species of a gram positive bacteria in a sample comprising

providing a sample suspected of containing said gram positive bacteria;

hybridizing a specific probe for a *sodA* gene or a fragment thereof to nucleic acids from said microorganism; and

detecting the presence or absence of hybridization.

2. The method according to claim 1, further comprising amplification of said *sodA* gene from the microorganism prior to said hybridizing.

3. The method according to claim 1, wherein said microorganism is selected from the group consisting of *Enterococci, Abiotrophia*, *Streptococci* and *Staphylococci*.

4.The method according to claim 3, wherein said microorganism is an *Enterococci* and is selected from the group consisting of *E. avium, E. casseliflavus, E. cecorum, E. columbae, E. dispar, E. durans, E. faecalis, E. faecium, E. flavescens, E. gallinarum, E. hirae, E. malodoratus, E. mundtii, E. pseudoavium, E. raffinosus, E. saccharolyticus, E. seriolicida, E. solitarius,* and *E. sulfureus*

5. The method of claim 1, wherein said specific probe is selected from the group consisting of SEQ ID NOS:1-94.

6. The method according to claim 3, wherein said microorganisms is a *Staphyloccus* and is selected from the group consisting of *S.arlettae, S. auricularis, S. capitis* subspecies *capitis, S. capitis* subspecies *ureolyticus, S. caprae, S. carnosus* subspecies *carnosus, S. carnosus* subspecies *utilis, S. chromogenes, S cohnii* subspecies *cohnii, S. cohnii* subspecies *urealyticum, S. condimenti, S. delphini, S epidermidis, S. equorum, S felis, S. gallinarum, S. haemolyticus, S. hominis* subspecies *hominis, S. hominis* subspecies *novobiosepticus, S. hyicus, S. intermedius, S. kloosii, S. lentus, S. lugdunensis, S. luntae, S. muscae, S. pasterui, S. piscifermentans, S. pulvereri, S. saccharolyticus, S. saprophyticus* subspecies *bovis, S. saprophyticus* subspecies *saprophyticus, S schleiferi* subspecies *coagulans, S. schleiferi* subspecies *schleiferi, S. sciuri* subspecies *carnaticus, S. sciuri* subspecies *sciuri, S. simulans, S vitulinus, S. warneri,* and *S. xylosus.*

7. A polynucleotide specifically hybridizing to an Enterococcus microorganism, wherein said polynucleotide comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1 specific for *E. avium,* SEQ ID NO:2 specific for *E. casseliflavus,* SEQ ID NO:3 specific for *E. cecorum,* SEQ ID NO:4 specific for *E. columbae,* SEQ ID NO:5 specific for *E. dispar,* SEQ ID NO:6 specific for *E. durans,*SEQ ID NO:7 specific for *E. faecalis,* SEQ ID NO:8 specific for *E. faecium,* SEQ ID NO:9 specific for *E. flavescens,* SEQ ID NO:10 specific for *E.*

*gallinarum*, SEQ ID NO:11 specific for *E. hirae*, SEQ ID NO:12 specific for *E. malodoratus*, SEQ ID NO:13 specific for *E. mundtii*, SEQ ID NO:14 specific for *E. pseudoavium*, SEQ ID NO:17 specific for *E. raffinosus*, SEQ ID NO:15 specific for *E. saccharolyticus*, SEQ ID NO:18 specific for *E. seriolicida*, SEQ ID NO:16 specific for *E. solitarius*, and SEQ ID NO:19 specific for *E. sulfureus*, and fragments thereof.

8. A polynucleotide specifically hybridizing to a microorganism of the genus *Enterococci*, wherein said polynucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS:21-36 specific for species in the *Enterococci*, and fragments thereof.

9. A polynucleotide specifically hybridizing to a microorganism of the genus *Lactococcus garvieae*, wherein said polynucleotide comprises a nucleotide sequence of SEQ ID NO: 20, or a fragment thereof.

10. A polynucleotide specifically hybridizing to a microorganism of the genus *Streptococcus*, wherein said polynucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS:37-50 specific for species in the *Streptococci*, and fragments thereof.

11. A polynucleotide specifically hybridizing to a microorganism of the genus *Abiotrophia*, wherein said polynucleotide comprises a nucleotide sequence selected

from the group consisting of SEQ ID NOS:51-53 specific for species in the *Abiotrophia*, and fragments thereof.

12. A polynucleotide specifically hybridizing to a microorganism of the genus *Staphlococcus*, wherein said polynucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS:54-93 specific for species in the *Staphlococcus*, and fragments thereof.

13. A DNA chip comprising at least one polynucleotide or a fragment thereof according to claims 7, 8, 9, 10, 11, or 12.

14. The method according to claim 1, wherein said fragment of *sodA* is $sodA_{int}$.

15. A method for the identification of a gram positive bacterial species selected from the group consisting of *Streptococci*, *Staphylococci*, *Abiotrophia* and *Enterococci* comprising

selecting a polynucleotide of about 425 to 445 bp comprised between two conserved domains of SOD gene said polynucleotide having flanking regions consisting in two oligonucleotidic sequences and being specific for the genus or the species to be detected;

hybridizing the DNA of the sample with the polynucleotide;

washing the hybridized sample;

visualizing the reaction of hybridization with an electric or electronic or calorimetric system.

16. A kit for the detection of a gram positive bacteria present in a sample containing at least a polynucleotide of SEQ ID NOS: 1-94.

17. A 400 bp polynucleotide sequence obtained after amplification of a DNA template from a sample by using a pair of primers SEQ ID NOS:95 and 96, wherein said pair of primers is specific for the SOD gene of a gram positive bacteria.

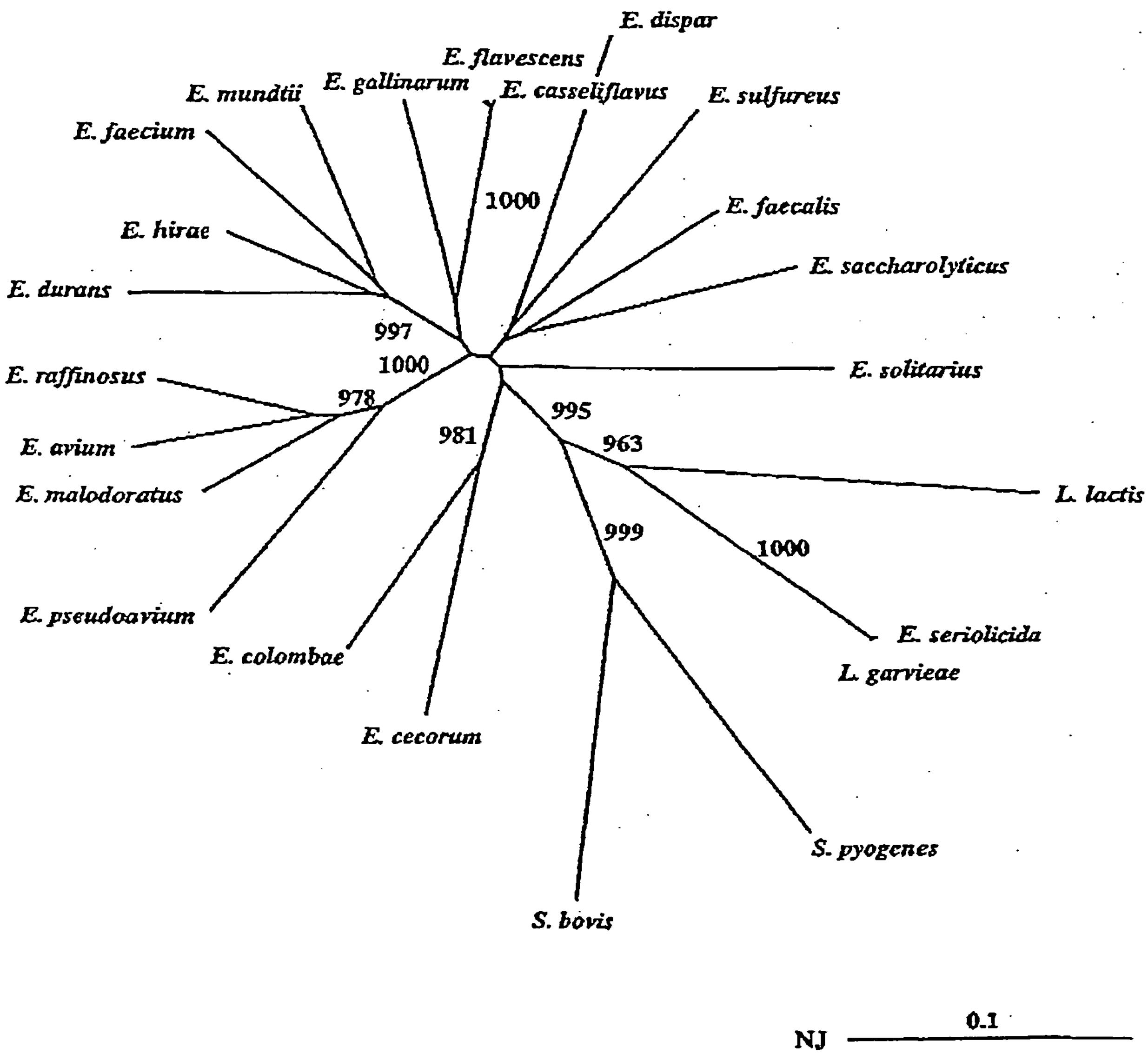
18. The method of Claim 15, wherein the polynucleotide is about 429bp and is specific for a *Staphylococi* species.

19. The method of Claim 15, wherein the polynucleotide is about 435 and is specific for *Streptococci* species.

20. The method of Claim 15, wherein the polynucleotide is about 438 bp and is specific for *Enterococci* species.

21. The method of Claim 15, wherein the polynucleotide is about 438 to 441 bp and is specific for Abiotrophia species.

FIGURE 1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E. saccharolyticus
E. durans
997
1000
E. solitarius
E. raffinosus
978
995
E. avium
981
963
L. lactis
E. malodoratus
999
1000
E. pseudoavium
E. seriolicida
E. colombae
L. garvieae
E. cecorum
S. pyogenes
S. bovis
NJ
0.1

FIGURE 2

| Strain | % of identity with: 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 *E. avium* | 74.0 | 67.4 | 71.9 | 70.3 | 70.3 | 73.7 | 69.9 | 74.2 | 75.1 | 75.6 | 71.0 | 80.1 | 72.6 | 74.0 | 85.4 | 87.9 | 60.7 | 67.1 |
| 2 *E. casseliflavus* | | 66.4 | 70.8 | 72.6 | 72.4 | 77.9 | 72.4 | 99.5 | 83.1 | 78.5 | 77.4 | 71.5 | 73.5 | 74.0 | 76.7 | 76.7 | 66.4 | 75.6 |
| 3 *E. cecorum* | | | 78.8 | 72.4 | 66.0 | 68.7 | 66.2 | 66.9 | 67.4 | 70.3 | 64.2 | 65.5 | 71.7 | 65.8 | 67.6 | 66.0 | 62.8 | 68.7 |
| 4 *E. colombae* | | | | 69.4 | 68.9 | 71.7 | 69.2 | 70.8 | 72.6 | 73.1 | 68.7 | 69.6 | 72.1 | 72.8 | 73.3 | 71.9 | 67.1 | 69.2 |
| 5 *E. dispar* | | | | | 70.3 | 77.4 | 68.7 | 72.8 | 72.1 | 73.5 | 72.8 | 70.5 | 71.0 | 69.4 | 72.1 | 70.3 | 62.6 | 74.9 |
| 6 *E. durans* | | | | | | 73.1 | 81.3 | 72.4 | 76.3 | 84.9 | 80.1 | 69.6 | 72.8 | 70.5 | 71.5 | 73.3 | 62.1 | 73.7 |
| 7 *E. faecalis* | | | | | | | 72.6 | 78.3 | 77.6 | 77.9 | 72.4 | 71.2 | 78.8 | 73.5 | 77.9 | 75.1 | 67.1 | 76.5 |
| 8 *E. faecium* | | | | | | | | 72.4 | 77.2 | 83.1 | 81.7 | 67.4 | 72.6 | 69.4 | 71.9 | 71.9 | 62.1 | 72.1 |
| 9 *E. flavescens* | | | | | | | | | 83.1 | 78.3 | 77.2 | 72.1 | 72.8 | 74.4 | 77.2 | 76.9 | 65.8 | 74.9 |
| 10 *E. gallinarum* | | | | | | | | | | 80.8 | 78.5 | 73.3 | 76.0 | 73.5 | 77.4 | 75.1 | 66.2 | 76.7 |
| 11 *E. hirae* | | | | | | | | | | | 83.6 | 71.5 | 73.3 | 73.5 | 77.4 | 76.7 | 63.5 | 75.3 |
| 12 *E. mundtii* | | | | | | | | | | | | 70.8 | 69.4 | 69.6 | 71.9 | 73.5 | 63.9 | 74.4 |
| 13 *E. pseudoavium* | | | | | | | | | | | | | 70.5 | 69.2 | 81.7 | 80.4 | 62.8 | 65.3 |
| 14 *E. saccharolyticus* | | | | | | | | | | | | | | 72.4 | 75.1 | 71.0 | 62.1 | 76.7 |
| 15 *E. solitarius* | | | | | | | | | | | | | | | 74.2 | 72.8 | 64.8 | 72.1 |
| 16 *E. malodoratus* | | | | | | | | | | | | | | | | 87.9 | 63.5 | 70.5 |
| 17 *E. raffinosus* | | | | | | | | | | | | | | | | | 64.6 | 67.6 |
| 18 *E. seriolicida* | | | | | | | | | | | | | | | | | | 61.6 |
| 19 *E. sulfureus* | | | | | | | | | | | | | | | | | | |

SEQUENCE LISTING

<110> Institut PASTEUR
Institut National de la Santé et de la Recherche Medicale INSERM

<120> Method for detecting and identifying a gram positive bacteria in a sample

<130> D19553

<150> US 60/205,237
<151> 2000-05-19

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gtgggtgaat taaaagctgc tattgacgaa acttttggta gcttcgatga attcaaggca 300

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gttggtgaat taaaagctgc cattgaagaa gcttttggta gctttgatga atttaaggct 300

caatttaaag cagcagcagc agctcgcttt ggctctggct gggcatggtt agtagtcgat 360

aacggtaaat tagcaattat ttcaacagca aaccaagata atccattaat ggaaggtaaa 420

gtacctgtcg ttggctta 438

<210> 5
<211> 438
<212> DNA
<213> Enterococcus dispar

<220>
<223> CIP 103646 T (ATCC 51266)

<400> 5

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attgcctata tggatgaaat tcctgctgat attcgtactg ctgttcaaaa taatggtggt    180

ggacatgcaa accatacatt cttttgggaa attatggcac caaatgctgg tggaacgcca    240

actggagctt taaaggatgc tattgacgaa acatttggtt cttttgaaga tttcaaaagt    300

gaatttaaaa ctgctgcgac aggacgtttc ggttctggtt gggcatggtt agtggtaaat    360

aacggtaaat tatctatcat gtcaactgcg aaccaagatt caccattaat ggaaggcaaa    420

actcccatta tcggttta                                                  438
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<210> 6
<211> 438
<212> DNA
<213> Enterococcus durans

<220>
<223> CIP 55.125 T (ATCC 19432)

<400> 6

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ctttctgata tggacgcgat tcctactgat attaagacag cggtacaaaa caatggcggt    180

ggacatgcaa accattcatt tttctggaaa atcatggcac ctaatgcagg tggcgaacca    240

acaggcgaaa tcaaagaagc gattgatgaa gcttttggtg atttcgcaac attcaaagaa    300

gagttcaaga aagcggctgc cggacgcttt ggatcaggtt gggcatggtt agtattggaa    360

gatggtaaat tggcaatcac ttctacagca aaccaagatt ctccattgat gacaggccaa    420

acacctatct taggatta                                                  438
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<210> 7
<211> 438
<212> DNA
<213> Enterococcus faecalis

<220>
<223> CIP 103015 T (ATCC 19433)

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atttcagata tgaatgctat tcctgaagat atccgtacag ccgttcgtaa caatggtggc 180

ggtcacgcaa accaaacatt cttctgggaa attatggcac caaatgctgg tggacaacca 240

actggcgcta ttaaagaagc aatcgatgaa acatttggta gctttgatga aatgaaagct 300

gctttcaaaa cagctgcaac tggccgcttt ggttcaggtt gggcttggtt agttgtgaat 360

aacggtaaat tagaaatcac ttcaacacca aaccaagatt caccattaat ggatggccaa 420

acacctgttt taggtctt 438

<210> 8
<211> 438
<212> DNA
<213> Enterococcus faecium

<220>
<223> CIP 103014 T (ATCC 19434)

<400> 8
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aatttaaatt cagcaattga gaaataccca gaattaggcg aaaaaacaat agaagaatta 120

ttatctgata tggacgctat tccaacagat atcaagacag ctgtacgtaa caatggtggc 180

ggacatgcta accattcatt tttctgggaa atcatggcac caaatgctgg tggcgaacct 240

acaggagaaa taaaagaagc gattaatgaa gcttttggtg atttttcttc ttttaaagaa 300

gaattcaaaa aagcagccgc tggacgattt ggttctggat gggcttggct tgtaatggaa 360

aatggaaaat tagctattac ctctactgca aatcaagatt ctccattgat ggaaggaaag 420

acaccaattc taggtttg 438

<210> 9
<211> 438
<212> DNA
<213> Enterococcus flavescens

<220>
<223> CIP 103525 T (ATCC 49996)

<400> 9
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aacttaaatg cagcgattga aaaacatcct gaattaggtg aaaaaaaagt tgaagaatta 120

ttagcagact tttcttctgt acctgaagat attcaaacag cggttcgcaa caatggcggc 180

ggccatgcta accacacgtt cttctgggaa atcttaggcc caaatgctgg tggcgaacct 240

actggggcaa tcaaagaggc aattgaagaa acattcggca gctttgaaga ctttaaagaa 300

gaatttaaaa ctgctgcaac tggacgtttt ggttcaggtt gggcatggtt agtcgttaaa 360

gacggtaaac tagcaatcac ttcaacagcg aatcaagatt caccattgat ggatggtcaa 420

acacctgttt taggttta 438

<210> 10
<211> 438
<212> DNA
<213> Enterococcus gallinarum

<220>
<223> CIP 103013 T (ATCC 49573)

<400> 10
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aatttgaatg cagcaattga aaaacatcct gaattaggtg aaaaatcagt tgaagaatta 120

cttgctgatt ttgattcggt tcctgaagac atcaaaacag ctgtccgtaa taacggtggt 180

ggtcatgcaa atcacagctt tttctgggaa atcttggcac caaatgctgg tggtgaacca 240

acaggagcca tcaaagaagc catcgaagaa acatttggca gctttgctga tttcaaagaa 300

gaattcaaaa cagcagcaac tggccgcttt ggttctggct gggcttggtt agtcatcaaa 360

gatggtaaat tagcgatcac ttccactgcg aaccaagatt caccattaat ggatggtcaa 420

acgccagttt taggttta 438

<210> 11
<211> 438
<212> DNA
<213> Enterococcus hirae

<220>
<223> CIP 53.48 T (ATCC 8043)

<400> 11
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ctttctgata tggatgctgt ccctacagat atcaagactg ctgtacgtaa taatggtggc 180

ggacatgcaa accattcttt cttctggaaa atcatggcac caaatgctgg tggcgaacca 240

actggtgcaa ttaaagaagc gattgatgaa gcctttggtg attttgcaac atttaaggaa 300

gaatttaaaa aagctgcagc tggccgtttt ggttcaggtt gggcttggtt agtgatggaa 360

aatggtaaat tagcgatcac ttcaacagcc aatcaagatt caccattaat ggaaggcaaa 420

acacctattt taggttta 438

<210> 12
<211> 438
<212> DNA
<213> Enterococcus malodoratus

<220>
<223> CIP 103012 T (ATCC 43197)

<400> 12
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aatttaaatg ctgcgattga aaaatatcca gaattagcag aacaatcagt ggaagaatta 120

gtaacgaact tgaatgaagt gccagaagat attcgtacgg ctgttcgcaa caatggcgga 180

ggtcatgcaa atcatagttt cttctggaaa atcatggcgc caaatgctgg cggaaaacca 240

acaggtgcga tcaaagatgc aattgatgaa gcattcggca gctttgaaaa aatgaaagaa 300

gaattcaaaa cagctgcaac tggccgcttt ggttctggct gggcttggct agtcttgaac 360

aatggtaaat tagaaattac ttcaacacca aatcaagata acccattaac agatggtaaa 420

acaccaatta ttggttta 438

<210> 13
<211> 438
<212> DNA
<213> Enterococcus mundtii

<220>
<223> CIP 103010 T (ATCC 43186)

<400> 13
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aacttaaatg cagcgatcga aaaatatcct gaactaggtg gaaaaacaat agaagaattg 120

gtttcagaca tggatgctat tccatctgac attcaaactg ctgtacgtaa taatggtggt 180

ggacatgcga accattcatt cttctggaaa atcatggcac caaatgctgg tggcgaacca 240

acaggagcaa tcaaagacgc aattaatgaa acattcggcg attttgcaac attcaaagaa 300

gaattcaaaa aagcagcagc aggacgtttc ggttctggct gggcttggtt agtacttgaa 360

gatggcaaac ttgccatcac ttctactgcc aaccaagatt caccattgat ggaaggcaag 420

aaacctgttc taggttta 438

<210> 14
<211> 438
<212> DNA
<213> Enterococcus pseudoavium

<220>
<223> CIP 103647 T (ATCC 49372)

<400> 14
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aatttgaatg tagcaattga aaaatatcct gaactagcgg agcaatctgt tgaggattta 120

gttgcaaact taaatgagtt gcctgaagat attcagacgg ctgttcgtaa caatggcggt 180

ggtcatgcga accatagctt tttctggaag atcatggcac caaacgcggg tggtgcgcca 240

actggtgcga tcaaagacgc cattgacgaa gctttcggcg gctttgaaaa aatgaaagaa 300

gaattcaaac ttgctgcgac aggacgtttt ggttctggtt gggcttggtt agtttggaac 360

aatggcaagt tggaaattac gtcaactgct aatcaagaca atccattgac tgacgggaaa 420

acaccaatca ttggctta 438

<210> 15
<211> 438
<212> DNA
<213> Enterococcus saccharolyticus

<220>
<223> CIP 103246 T (ATCC 43076)

<400> 15
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aacttaaatg cagcagttga aaaatatcct gaattaggcg aaaaatctgt agaagattta 120

atttctgatt tagcagcagt tcctgaagat attcgcacag ccgtacgcaa caatggtggt 180

ggacatgcaa accatacatt cttttgggaa attatggcac caaacgctgg tggcgaacct 240

gtaggcgagc taaaagcagc gattgacgaa aaatttggta gctttgatgc attcaaagca 300

gaatttaaag cagcagcgac tagccgattt ggttctggtt gggcttggtt agctttaaat 360

aatgggttat tagaagtgac ttctacacca aatcaagatt ctccattaat ggatggtcgt 420

acaccaattg ttggtttta 438

<210> 16
<211> 438
<212> DNA
<213> Enterococcus solitarius

<220>
<223> CIP 103330 T (NCTC 12193)

<400> 16
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aacttaaatg cagcgattga aaaacatcct gaattaggcg aaaaatcagt ggaagaccta 120

atggcagatc ttgatagtgt ccctgaagat atttttacag cagtacgtaa taacggcggt 180

ggacatgtaa atcattcttt cttctggaag attttatctc cagatggagg cggtgaacca 240

accggtgcat taaaagatgc gattgatcaa gaatttggca gttttgatgc ttttaaagat 300

gaatttaagg cagctgccac cggtcgtttt ggttctggct gggcttggtt agttttagat 360

aacggcaaat taaaaattac ttcgacgcca aaccaagatt ctccattgac agatggacaa 420

attcctatta ttggctta 438

<210> 17
<211> 438
<212> DNA
<213> Enterococcus raffinosus

<220>
<223> CIP 103329 T (ATCC 49427)

<400> 17
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aacttgaatg ctgcgattga aaaatatcca gaattaggcg aacaatcaat cgaagaatta 120

gtgacgaact tgaatgaagt tcctgaagac attcgtacag cggtacgtaa taatggcggc 180

ggacatgcga accacagctt cttctggaaa atcatggcgc ctaatgctgg cggcgaacca 240

acaggtgcga tcaaagaagc aattgatcaa gctttcggca gctttgagaa aatgaaggaa 300

gaattcaaga cagcggcaac aggacgtttt ggttctggtt gggcatggtt ggtattgaac 360

aacggtaaat tagaaattac atcaaccgcg aatcaagata gcccattgac tgatggcaaa 420

acaccaatta ttggttta 438

<210> 18
<211> 438
<212> DNA
<213> Enterococcus seriolicida

<220>
<223> CIP 104369 T (ATCC 49156)

<400> 18
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aatcttaatg cagcgattga aaaacaccca gaattctttg ataaaactgt tgaagaatta 120

gtggcttatt tggaccgttt gccagaagac attcgtgttg cggtacgtaa caacggtgga 180

ggacacttga accacacaat gttctgggaa tggctcgctc caaatgcagg tggtgcacca 240

acaggtgata tcgctgcagc aatcgatgaa gcttttggtt catttgacga cttcaaagct 300

gaatttaaag cagctgctac aggacgtttc ggttcaggtt gggcttggtt agttcttgat 360

tacggtaaac ttaaggttgt ttccacagca aaccaagata acccaatttc tgatggccaa 420

attccagtgc ttggtctt 438

<210> 19
<211> 438
<212> DNA
<213> Enterococcus sulfureus

<220>
<223> CIP 104373 T (DSM 6905)

<400> 19
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aacttaaatg cagcggttga aaaatatcct gaattagcag aaaaatcagt ggaagactta 120

atcgcagata tggatgcaat cccaagtgat attcaaacag cagtacgtaa taatggtggt 180

ggccatgcca atcatagttt cttctgggaa atcttgacac caaatgctac tgaagaacca 240

gtaggcgaat taaaaacagc gatcgaagat acatttggat ctttagatgc attaaaagaa 300

gaatttaaaa aagcagcaac tggccgtttt ggttcaggtt gggcttggtt agtagtaaaa 360

gacggtaaat tagccgtaac gtctacagca aaccaagatt caccattaat agaaggccaa 420

actcctgttt taggttta 438

<210> 20
<211> 438
<212> DNA
<213> Lactococcus garvieae

<220>
<223> CIP 102507 T (DSM20684)

<400> 20
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aatcttaatg cagcgattga aaaacaccca gaattctttg ataaaactgt tgaagaatta 120

gtggcttatt tggaccgttt gccagaagac attcgtgttg cagtacgtaa caacggtgga 180

ggacacttga accacacaat gttctgggaa tggctcgctc caaatgcagg tggtgcacca 240

acaggtgata tcgctgcagc aatcgatgaa gcttttggtt catttgacga cttcaaagct 300

gaatttaaag cagctgctac aggacgtttc ggttcaggtt gggcttggtt agttcttgat 360

tacggtaaac ttaaagttgt ttccacagca aaccaagata acccaatttc tgatggccaa 420

attccagtgc ttggtctt 438

<210> 21
<211> 438
<212> DNA
<213> Enterococcus faecalis

<220>
<223> CIP 105042

<400> 21
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aacttaaacg cagcgattga aaaacatcca gaattaggcg aaaaatctgt agaagaccta 120

atttcagata tgaatgctat tcctgaagat atccgcacag ccgttcgtaa caatggtggc 180

gggcacgcaa accatacatt cttctgggaa attatggcac caaatgctgg tggacaacca 240

actggcgcta ttaaagaagc aatcgatgaa acatttggca gctttgatga aatgaaagct 300

gctttcaaaa cagctgcaac tggccgcttt ggttcaggtt gggcttggtt agttgtgaat 360

aacggtaaat tagaaatcac ttctacacca aaccaagatt caccattaat ggatggccaa 420

acacctgttt taggtctt 438

<210> 22
<211> 438
<212> DNA
<213> Enterococcus faecalis

<220>
<223> NEM1616 (Clinical isolate)

<400> 22
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aacttaaacg cagcgattga aaaacatcca gaattaggcg aaaaatctgt agaagaccta 120

atttcagata tgaatgctat tcctgaagat atccgtacag ccgttcgtaa caatggtggc 180

ggtcacgcaa accatacatt cttctgggaa attatggcac caaatgctgg tggacaacca 240

actggcgcta ttaaagaagc aatcgatgaa acatttggta gctttgatga aatgaaagct 300

gctttcaaaa cagctgcaac tggccgcttt ggttcaggtt gggcttggtt agttgtgaat 360

aacggtaaat tagaaatcac ctcaacacca aaccaagatt caccattaat ggatggccaa 420

acacctgttt taggtctt 438

<210> 23
<211> 438
<212> DNA
<213> Enterococcus faecalis

<220>
<223> NEM1617 (Clinical isolate)

<400> 23
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aacttaaacg cagcgattga aaaacatcca gaattaggcg aaaaatctgt agaagaccta 120

atttcagata tgaatgctat tcctgaagat atccgcacag ccgttcgtaa caatggtggc 180

gggcacgcaa accatacatt cttctgggaa attatggcac caaatgctgg cggacaacca 240

actggcgcta ttaaagaagc aatcgatgaa acatttggca gctttgatga aatgaaagct 300

gctttcaaaa cagctgcaac tggccgcttt ggttcaggtt gggcttggtt agttgtgaat 360

aacggtaaat tagaaatcac ttctacacca aaccaagatt caccattaat ggatggccaa 420

acacctgttt taggtctt 438

<210> 24
<211> 438
<212> DNA
<213> Enterococcus durans

<220>
<223> NEM1618 (Clinical isolate)

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aatttaaacg cagctattga aaagtatcca gaattaggcg aaaaatcagt ggaagaattg 120

ctttctgata tggacgcgat tcctactgat attaagacag cggtacaaaa caatggtggt 180

ggacatgcaa accattcatt tttctggaaa atcatggcac ctaatgcagg tggcgaacca 240

acaggggaaa tcaaagaagc gattgatgaa gcttttggtg atttcgcaac atttaaagaa 300

gagttcaaga aagcggctgc cggacgcttt ggatcaggtt gggcatggtt agtattggaa 360

gatggtaaat tggcaatcac ttctacagca aaccaagatt ctccattgat gacaggccaa 420

acacctatct taggatta 438

<210> 25
<211> 438
<212> DNA
<213> Enterococcus durans

<220>
<223> NEM1619 (Clinical isolate)

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aatttaaacg cagctattga aaagtatcca gaattaggcg aaaaatcagt ggaagaattg 120

ctttctgata tggacgcgat tcccactgat attaagacag cggtacaaaa caatggtggt 180

ggacatgcaa accattcatt tttctggaaa atcatggcac ctaatgcagg tggcgaacca 240

acaggcgaaa tcaaagaagc gattgatgaa gcttttggtg atttcgcaac atttaaagaa 300

gagttcaaga aagcggctgc cggacgcttt ggatcaggtt gggcatggtt agtattggaa 360

gatggtaaat tggcaatcac ttctacagca aaccaagatt ctccattgat gacaggccaa 420

acacctatct taggatta 438

<210> 26
<211> 438
<212> DNA
<213> Enterococcus durans

<220>
<223> NEM1620 (Clinical isolate)

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aatttaaacg cagctattga aaagtatcca aaattaggcg aaaaatcagt ggaagaattg 120

ctttctgata tggacgcgat tcctactgat attaagacag cggtacaaaa caatggtggt 180

ggacatgcaa accattcatt tttctggaaa atcatggcac ctaatgcagg tggcgaacca 240

acaggcgaaa tcaaagaagc gattgatgaa gcttttggtg atttcgcaac atttaaagaa 300

gagttcaaga aagcggctgc cggacgcttt ggatcaggtt gggcatggtt agtattggaa 360

gatggtaaat cggcaatcac ttctacagca aaccaagatt ctccattgat gacaggccaa 420

acacctatct taggatta 438

<210> 27
<211> 438
<212> DNA
<213> Enterococcus hirae

<220>
<223> NEM1621 (Clinical isolate)

<400> 27
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aatttaaatg cagcgattga aaaacatcca gaactaggtg aaaaaacaat cgaagaacta 120

ctttctgata tggatgctgt ccctacagat atcaagactg ctgtacgtaa taatggtggc 180

ggacatgcaa accattcttt cttctggaaa atcatggcac caaatgctgg tggcgaacca 240

actggtgcaa ttaaagaagc gattgatgaa gcctttggtg attttgcaac atttaaggaa 300

gaatttaaaa aagctgcagc tggccgtttt ggttcaggtt gggcttggtt agtgatggaa 360

aatggtaaat tagcgatcac ttcaacagcc aaccaagatt caccattaat ggaaggcaaa 420

acacctattt taggttta 438

<210> 28
<211> 438
<212> DNA
<213> Enterococcus hirae

<220>
<223> NEM1622 (Clinical isolate)

<400> 28
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aatttaaatg cagcgattga aaaacatcca gaactaggtg aaaaaacaat cgaagaacta 120

ctttctgata tggatgctgt ccctacagat atcaagactg ctgtacgtaa taatggtggc 180

ggacatgcaa accattcttt cttctggaaa atcatggcac caaatgctgg tggcgaacca 240

actggtgcaa ttaaagaagc gattgatgaa gcctttggtg attttgcaac atttaaggaa 300

gaatttaaaa aagctgcggc tggccgtttt ggttcaggtt gggcttggtt agtgatggaa 360

aatggtaaat tagcgatcac ttcaacagcc aaccaagatt caccattaat ggaaggcaaa 420

acacctattt taggttta 438

<210> 29
<211> 438
<212> DNA
<213> Enterococcus casseliflavus/Enterococcus flavescens

<220>
<223> NEM1623 (Clinical isolate)

<400> 29
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ttagcagact tttcttctgt acctgaagat attcaaacag cggttcgcaa caatggcggc 180

ggccatgcta accacacatt cttctgggaa atcttaggcc caaatgctgg tggcgaacct 240

actggggcaa tcaaagaggc aattgaagaa acattcggca gctttgaaga ctttaaagaa 300

gaatttaaaa ctgctgcaac tggacgtttt ggttcaggtt gggcatggtt agtcgttaaa 360

gacggtaaac tagcaatcac ttcaaccgcg aatcaagatt caccattgat ggatggtcaa 420

acacctgtat taggttta 438

<210> 30
<211> 438
<212> DNA
<213> Enterococcus faecium

<220>
<223> NEM1624 (Clinical isolate)

<400> 30
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aatttaaatg cagcaattga gaaataccca gaattaggcg aaaaaacaat agaagaatta 120

ttatctgata tggacgctat tccaacagat atcaagacag ctgtacgtaa caatggtggc 180

ggacatgcta accattcatt tttctgggaa atcatggcac caaatgctgg tggcgaacct 240

acaggagaaa taaaagaagc gattaaagaa gcttttggtg atttttcttc ttttaaagaa 300

gaattcaaaa aagcagccgc tggacgattt ggttctggat gggcttggct tgtaatggaa 360

aatggaaaat tagctattac ctctactgca aatcaagatt ctccattgat ggaaggaaag 420

acaccaattc taggtttg 438

<210> 31
<211> 438
<212> DNA
<213> Enterococcus faecium

<220>
<223> NEM1625 (Clinical isolate)

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ttatctgata tggacgctat tccaacagat atcaagacag ctgtacgtaa caatggtggc 180

ggacatgcta accattcatt tttctgggaa atcatggcac caaatgctgg tggcgaacct 240

acaggagaaa taaaagaagc gattaatgaa gcttttggtg atttttcttc ttttaaagaa 300

gaattcaaaa aagcagccgc tggacgattt ggttctggat gggcttggct tgtaatggaa 360

aatggaaaat tagctattac ctctactgca aatcaagatt ctccattgat ggaaggaaag 420

acaccaattc taggtttg 438

<210> 32
<211> 438
<212> DNA
<213> Enterococcus faecium

<220>
<223> NEM1626 (Clinical isolate)

<400> 32
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aatttaaatg cagcaattga gaaataccca gaattaggcg aaaaaacaat agaagaatta 120

ttatctgata tggacgctat tccaacagat atcaagacag ctgtacgtaa caatggtggc 180

ggacatgcta accattcatt tttctgggaa atcatggcac caaatgctgg tggcgaacct 240

acaggagaaa taaaagaagc gattaatgaa gcttttggtg atttttcttc ttttaaagaa 300

gaattcaaaa aagcagccgc tggacgattt ggttctggat gggcttggct tgtaatggaa 360

aatggaaaat tagctattac ctctactgca aatcaagatt ctccattgat ggaaggaaag 420

acaccaattc taggtttg 438

<210> 33
<211> 438
<212> DNA
<213> Enterococcus faecium

<220>
<223> NEM1627 (Clinical isolate)

<400> 33
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aatttaaatg cagcaattga gaaataccca gaattaggcg aaaaaacaat agaagaatta 120

ttatctgata tggacgctat tccaacagat atcaagacag ctgtacgtaa caatggtggc 180

ggacatgcta accattcatt tttctgggaa atcatggcac caaatgctgg tggcgaacct 240

acaggagaaa taaaagaagc gattaatgaa gcttttggtg atttttcttc ttttaaagaa 300

gaattcaaaa aagcagccgc tggacgattt ggttctggat gggcttggct tgtaatggaa 360

aatggaaaat tagctattac ctctactgca aatcaagatt ctccattgat ggaaggaaag 420

acaccaattc taggtttg 438

<210> 34
<211> 438
<212> DNA
<213> Enterococcus faecium

<220>
<223> NEM1628 (Clinical isolate)

<400> 34
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aatttaaatt cagcaattga gaaataccca gaattaggcg aaaaaacaat agaagaatta 120
ttatctgata tggacgctat tccaacagat atcaagacag ctgtacgtaa caatggtggc 180
ggacatgcta accattcatt tttctgggaa atcatggcac caaatgctgg tggcgaacct 240
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gaattcaaaa aagcagccgc tggacgattt ggttctggat gggcttggct tgtaatggaa 360
aatggaaaat tagctattac ctctactgca aatcaagatt ctccattgat ggaaggaaag 420
acaccaattc taggtttg 438

<210> 35
<211> 438
<212> DNA
<213> Enterococcus gallinarum

<220>
<223> NEM1629 (Clinical isolate)

<400> 35
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aatttgaatg cagcaattga aaaacatcct gaattaggtg aaaaatcagt tgaagaatta 120
cttgctgatt ttgattcggt gcctgaagac atcaaaacag ctgtccgtaa taacggtggt 180
ggtcatgcaa atcacagctt tttctgggaa atcttggcac caaatgctgg tggtgaacca 240
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gaattcaaaa cagcagcaac tggccgcttt ggttctggct gggcttggtt agtcatcaaa 360
gatggtaaat tagcgatcac ttcaactgcg aaccaagatt caccattaat ggacggtcaa 420
acgccagttt taggctta 438

<210> 36
<211> 438
<212> DNA
<213> Enterococcus avium

<220>
<223> NEM1630 (Clinical isolate)

<400> 36
tatatcgatg ttgaaacgat gcatttgcat catgacaaac accataacac ttatgtaaca 60
aatttaaatg ctgcgattga aaaatatccg gaattagaag aacagtcaat tgaagagcta 120

atgaaaaact taaatgaagt tcctgaggac attcgtacgg ctgtacgtaa taacggcggc 180

ggacatgcta accacagctt cttctggaaa attatggctc caaatgctgg tggtgaacct 240

acaggcgcga ttaaggacgc aattgatcaa gcatttggca gctttgaaaa aatgaaggaa 300

gaattcaaga ctgcagcaac tggtcgtttt ggttctggct gggcatggtt agtattgaac 360

aatggaaaat tagaaattac ttctactgca aatcaagaca gcccattaac tgatggaaaa 420

acaccgatca ttggctta 438

<210> 37
<211> 435
<212> DNA
<213> Streptococcus difficilis

<220>
<223> CIP 103768 T (ATCC 51487)

<400> 37
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aatgcaaatg ctgctcttga gaaacatcct gaaattggag aagacttaga ggcgctctta 120

gctgatgttt ctcaaattcc agaagatatt cgtcaggcag tcatcaataa cggtggtgga 180

catcttaacc acgctctttt ctgggaattg atgtcaccag aagaaactca aatttcaaaa 240

gagttatctg aagacattga tgcaactttt ggttcatttg aagactttaa agctgctttc 300

acagcagcag caacaggacg ttttggttca ggttgggctt ggcttgttgt taatgctgaa 360

ggcaaacttg aagtgctttc aactgccaat caagatactc caattatgga aggtaagaaa 420

ccaattttag ggctt 435

<210> 38
<211> 435
<212> DNA
<213> Streptococcus ferus

<220>
<223> CIP 103225 T (ATCC 33477)

<400> 38
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aacgcaaatg cagcccttga aaaacaccca gaaatcggtg acgatttaga aaaattgttg 120

gccgatgttg agtctattcc agaagatatc cgccaggctt tgattaataa tggcggaggc 180

catctgaatc atgcgctttt ctgggagttg ctgtcaccag aaaaaacaac catttcagct 240

gaactgaagg ctgatattga agctagtttt ggttcttttg accagtttaa agaggccttt 300

acaacggctg ctacaacacg ctttggttca ggctgggctt ggctcgttgt caatcaagaa 360

ggacagttag aggtggtttc aacagctaat caagacacac caatttcaca aggtttgaaa 420

cccatcttgg ttcta 435

<210> 39
<211> 435
<212> DNA
<213> Streptococcus gallolyticus

<220>
<223> CIP 105428 T (JCM 10005)

<400> 39
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aatgtaaatg cagcgcttga aaaacatcca gaaattggag aggatttgga agctttgttg 120

gcagatgttg acagtattcc agcagatatc cgtcaagcgg tgattaataa cggtggtggg 180

catttgaatc acgccctttt ctgggaattg ttatcgcctg aaaaacaaga accaacagcg 240

caagtgttgg ctgcgattga ggaagatttt ggctcatttg acgaattcaa agctgctttc 300

acgcaagctg cgacaactcg ctttgggtca gggtgggctt ggcttgtggt gaatgaaaat 360

ggcaaacttg aagtgctctc aacagctaat caagacacac caatttcaca aggaaaagca 420

ccaattttgg cactt 435

<210> 40
<211> 435
<212> DNA
<213> Streptococcus hyointestinalis

<220>
<223> CIP 103372 T (ATCC 49169)

<400> 40
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aatgtcaatg cggcccttga aaaacacact gaaatcggtg aagacttggt ggcacttttg 120

tctgacgtgg aaaaaatccc tgctgacatc cgtcaagccg ttatcaacaa cggcggagga 180

catctcaacc acgctctttt ctgggaattg atgacaccag aaaagacaga ggtttcagca 240

gaattgttag cagatattga agctactttt ggctcatttg acgctttcaa agacgctttc 300

tcagcagcag ctgcgactcg ctttggctca ggttgggctt ggcttgtcgt gaatgctgaa 360

ggaaaactcg aaattctctc aacagctaac caagacaacc ctatcatgga tggcaaacaa 420

cctatccttg gacta 435

<210> 41
<211> 435
<212> DNA
<213> Streptococcus hyovaginalis

<220>
<223> CIP 105517 T

<400> 41
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aatgctaatg ccgctttgga aaaacacccc gaacttggag atgacgttgc agcactctta 120

tcggatgttg acagcattcc agaagatatt cgccaagccc tcatcaataa tggcggtggt 180

caccttaacc acgcattgtt ctgggaactt ctttcaccag aaaagacaga aatcacagaa 240

gatgtcaagg ctgctattga tgacgctttt ggttcatttg acgccttcaa agaggccttt 300

acggcggcag caacaacacg ttttggttca ggttgggcat ggttagttgt taatgcagaa 360

ggaaaacttg aggtgacatc aactccaaac caagatactc cacttatgga tggtaacacg 420

ccaatccttg gttta 435

<210> 42
<211> 435
<212> DNA
<213> Streptococcus infantarius

<220>
<223> CIP 103233 T

<400> 42
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aatgcaaatg ctgctcttga aaaacaccct gaacttggag atgatttaga agttatcttg 120

gcagagcttg acaagattcc agcagatatt cgtcaagcgg tgattaacaa cggtggtggt 180

gctcttaacc actcactttt ctgggaattg ctatctcctg aaaaacaaga accaacagca 240

gatgtacttg cggcaattga agaagcattt ggctcatttg aagatttcaa aacagctttc 300

acgcaagcag cgacaactcg ctttggttca ggttgggctt ggcttgtcgt taacaaagat 360

ggcaaacttg aagtaacctc aactgctaac caagatactc cactttcaga aggtaagaaa 420

ccaattcttg ctctt 435

<210> 43
<211> 435
<212> DNA
<213> Streptococcus macacae

<220>
<223> CIP 102912 T (ATCC 35911)

<400> 43
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aatgctaatg ctgcattgga aaaacaccca gaaataggtg aagatttaga aggcttactg 120

gcagatgttg agaagattcc tgaggatatt cgtcaggctt tgattaataa tggcggcggt 180

catcttaacc actctctttt ttgggaattg ctttccccag aaaaaacaga aatcactgaa 240

gaagtggctg cagctattaa tgattctttt ggctcttttg acgcttttaa agaagcattt 300

acaactgctg cgacgactcg ctttggttct ggctgggctt ggctggttgt caaccgccaa 360

gggaagcttg aagtgatttc aacggctaat caagatacgc caatttcaca agggctaaag 420

ccaatcctag cgctt 435

<210> 44
<211> 435
<212> DNA
<213> Streptococcus macedonicus

<220>
<223> CIP 105683 T

<400> 44
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aatgtaaatg cagcgcttga aaaacatcca gaaattggag aggatttgga aactttgttg 120

gcagatgttg acagtattcc agcagatatc cgtcaagcgg tgattaataa cggtggtggg 180

catttgaatc acgccctttt ctgggaattg ttatcgcctg aaaaacaaga accaacagcg 240

caagtgctgg ctgcgattga ggaagctttt ggctcatttg acgaattcaa agctactttc 300

acgcaagctg cgacaactcg ttttgggtca ggttgggctt ggcttgtggt gaatgaaaat 360

ggcaaacttg aagtgctctc aacagctaat caagatacac caatttcaca aggaaaagca 420

ccaattttgg cactt 435

<210> 45
<211> 435
<212> DNA
<213> Streptococcus parauberis

<220>
<223> CIP 103956 T (DSM 6631)

<400> 45
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aatgccaatg ctgctttaga aaaacaccca gaaattggtg aagatctaga aactcttcta 120

gcagacgtgg aatctattcc ttcagatatt cgtcaagccc taattaataa tggtggtgga 180

catttgaatc acgcactatt ttgggaatta ttatctcctg agaatactga aatttcttca 240

gaagttgcat ctgcaattga tgaagcattt ggttcatttg atgcctttaa agaacaattc 300

acagctgcag caacaggacg ttttggttct ggatgggcat ggctagttgt aaataaagaa 360

ggtaaacttg aaattatgtc aactgctaat caagatacac caatttcatc aggattaaaa 420

ccaattttag gattg 435

<210> 46
<211> 435
<212> DNA
<213> Streptococcus phocae

<220>
<223> CIP 104665 T

<400> 46
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aacactaatg ctgctttgga aaaacaccct gaaatcggtg aggaccttga agcattgtta 120

gcagatgttg atgcgatacc agcagatatt cgtcaagctg tgataaataa cggtggtggg 180

catttgaatc atagcttgtt ctgggaatta ctgtctccag aaaagcaaga ggttactgct 240

gacgttgccg cagccattga cgaagcattt ggttcgtttg atgcttttaa agaacaattc 300

actgcagcag caacaggtcg ctttggatca ggttgggcat ggttagttgt caataaagaa 360

ggcaagcttg aaatcacgtc aactgctaac caagacacac caatctcaga tggtaaaaag 420

cctattttaa cgctt 435

<210> 47
<211> 435
<212> DNA
<213> Streptococcus ratti

<220>
<223> CIP 102509 T (ATCC 19645)

<400> 47
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gctgatgttc agcaaattcc ggaagatatc cgtcaggctc ttgttaacaa cggcggcggt 180

caccttaacc acgcactttt ctgggaactt ctgtcaccag aaaaaacaga gattactaaa 240

gaagtggctg cagcaattga cgaagctttt ggctcatttg aggcttttaa gacagctttc 300

actcaggcag cagcaacacg ctttggttca ggctgggctt ggctagttgt caacgcagaa 360

ggtaagcttg aagtaatgtc aacagccaac caagatacac cgatttcgca aggtttaaaa 420

ccaatcttgg ccctt 435

<210> 48
<211> 435
<212> DNA
<213> Streptococcus thoraltensis

<220>
<223> CIP 105518 T

<400> 48
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aacgcaaatg ctgctttgga aaaacaccct gaaatcggtg aagaccttga agctcttttg 120

tcagatgtca acagcattcc tgaagacatt cgtcaagcgc ttatcaacaa tggcggtgga 180

catcttaacc atgccctttt ctgggaactt ctttcaccag aaaaaacaga aattacagaa 240

gatgtgaaag cagccattga tgaagctttc ggttcatttg aagccttcca agaaaaattc 300

actacagcag ctacaacacg ctttggttca ggttgggctt ggttagttgt taacgctgaa 360

ggtaaactcg aggtcacatc aacaccaaac caagacactc cacttatgga aggtaaaaaa 420

ccaatccttg gactt 435

<210> 49
<211> 435
<212> DNA
<213> Streptococcus uberis

<220>
<223> CIP 103219 T (ATCC 19436)

<400> 49
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aatgccaatg ctgcgcttga aaaacatcca gaaattggtg aagatttggt ggcgttatta 120

tctgatgtgt catcaattcc agaagatatt cgtcaagctc ttatcaataa tggaggcgga 180

catcttaacc atgcactttt ttgggaactt ctttcacctg agaaaacaga aatcacttcg 240

gaagtagctt ctgctattga tgaagcattt ggttcttttg atgcatttaa agaaaaattt 300

acagcagcag caacgggacg ttttggatct ggttgggctt ggttagttgt caataaagaa 360

ggagaacttg aagtaacttc aactgcaaac caagatacac caatttctga aggtaaacag 420

cctattttgg gtctt 435

<210> 50
<211> 435
<212> DNA
<213> Streptococcus waius

<220>
<223> CIP 106079 T

<400> 50
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aatgtaaatg cagcgcttga aaaacatcca gaaattggag aggatttgga aactttgttg 120

gcagatgttg acagtattcc agcagatatc cgtcaagcgg tgattaataa cggtggtggg 180

catttgaatc acgccctttt ctgggaattg ttatcgcctg aaaaacaaga accaacagcg 240

caagtgctgg ctgcgattga ggaagctttt ggctcatttg acgaattcaa agctactttc 300

acgcaagctg cgacaactcg ttttgggtca ggttgggctt ggcttgtggt gaatgaaaat 360

ggcaaacttg aagtgctctc aacagctaat caagatacac caatttcaca aggaaaagca 420

ccaattttgg cactt 435

<210> 51
<211> 438
<212> DNA
<213> Abiotrophia adjacens

<220>
<223> CIP 103243 T (ATCC 49175)

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aatttaaacg cagcggtaga aaaacaccct gaattattcg aaaaaacagt tgaagaatta 120

gttagcgatt taaacgctgt tccagaagat atccgtgtag ctgttcgcaa caatggtggt 180

gggcatgcaa accatagctt attctggact caattatctc ttgatggtgc aaaagctcca 240

gaaggtgctt tattagcagc tatcaacgaa gcattcggaa gcttcgacga attcaaagca 300

gcattcgcac aagcagcagc aactcgtttt gggtctggtt gggcttggtt agttctttct 360

aacggaaaat tagaagtcgt ttctactcca aaccaagata accctctatc agaaggcaaa 420

actccattat taggatta 438

<210> 52
<211> 441
<212> DNA
<213> Abiotrophia defectives

<220>
<223> CIP 103242 T (ATCC 49176)

<400> 52
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aacttgaatg ccgccttaga aggtcacgca gacttggcag ctaagtctat cgaagactta 120

gtcgctaacc ttaaggattt acctgaaagc attcaaacag ctgtccgtaa caatggtggg 180

ggtcacttca accatagctt cttctgggaa agcctacaag cgccaagtgc agaagcagct 240

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gaagcttttg ctaaggcagc tgcgactcgc tttggttctg gttgggcttg gctcgtagac 360

cgtgacggtc acttagaagt cttatctact gctaaccaag acacaccttt agaattaggg 420

cttaagccac ttttaggttt a 441

<210> 53
<211> 438
<212> DNA
<213> Abiotrophia elegans

<220>
<223> CIP 105513 T (DSM 11693)

<400> 53
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aacttaaacg cagcagtaga aaaacaccct gctttatttg aaaaaagtgt ggaagaatta 120

gtagcagatt tagcatctgt accagaaggt attcgtggag ctgttcgtaa caatggtggt 180

ggacatgcaa accacagctt attctggaca gtaatttcac cgaatggtgg aggtcaacct 240

actggcgaat tagcagcagc aatcgatagc aaattcggtg ggtttgatgc gtttaaacaa 300

gcattctctc aagcagcagc aactcgtttc ggttctggtt gggcttggtt agttgtttca 360

aatggtgaat tagaagtagt ttctactcca aaccaagata acccattaac agatggtaaa 420

actccaattt taggatta 438

<210> 54
<211> 429
<212> DNA
<213> Staphylococcus arlettae

<220>
<223> CIP 103501 T (ATCC 43959)

<400> 54
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aaattaaatg cagcagtaga aggtactgat ttagaatcta aatcaattga agaaatcgtc 120

gctaacttag atagcgtacc tgaagatatt caaacagctg tgcgtaacaa tggtggagga 180

catatcaacc attcattgtt ctgggaatta ttaactccta actctgaaga aaaaggtact 240

gtagttgata aaattaaaga acaatggggt tctttagatg catttaaaga agaatttgca 300

aataaagctg cagcacgttt tggttcaggt tgggcatggt tagtagtaaa taacggtaac 360

ttagaaatcg ttactacacc taaccaagac aacccattaa ctgaaggtaa aacacctatt 420

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<210> 55
<211> 432
<212> DNA
<213> Staphylococcus auricularis

<220>
<223> CIP 103587 T (ATCC 33753)

<400> 55
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gctaatttag atagcgtacc tgaagatatt caaacagctg tacgaaataa tggtggtgga 180

cacttaaatc actcattatt ctgggaatta ttaactccta actctgaaga aaaaggtaca 240

gtcgtagata aaattaaaga acaatggggt tctttagacg atttcaaaaa agaatttgct 300

gacgctgcag cagctcgctt tggttcagac tggggttggc tcgttgtaaa tgctgaaggt 360

aaattagaaa tcactactac acctaaccaa gataacccaa ttacagaagg taaaacacct 420

attttaggta tt 432

<210> 56
<211> 429
<212> DNA
<213> Staphylococcus capitis subsp. Capitis

<220>
<223> CIP 81.53 T (ATCC 27840)

<400> 56
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aaattaaact cagcagttga aggaacagat ttagaagcta aatcaatcga agaaattgtt 120

gctaatttag atagcgtacc ttcagatatt caaactgcag tacgtaataa tggtggcggt 180

cacttaaacc actcattatt ctgggaatta ttatcaccaa attctgaaga aaaaggtgaa 240

gtagtagaca aaattaaaga acaatggggt tctttagatg aattcaaaaa agaatttgca 300

gataaagctg ctgcacgctt tggatctggt tgggcatggt tagtagtaaa taacggtcaa 360

ttagaaatcg ttactactcc aaaccaagat aacccattaa ctgaaggtaa aactccaatc 420

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<213> Staphylococcus capitis subsp. Ureolyticus

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gctaatttag atagcgtacc ttcagatatt caaactgcag tacgtaataa tggtggcggt 180

cacttaaacc actcattatt ctgggaatta ttatcaccaa attctgaaga aaaaggtgaa 240

gtagtagaca aaattaaaga acaatggggt tctttagatg aattcaaaaa agaatttgca 300

gataaagctg ctgcacgctt tggatctggt tgggcatggt tagtagtaaa taacggtcaa 360

ttagaaatcg ttactactcc aaaccaagat aacccattaa ctgaaggtaa aactccaatc 420

ttaggttta 429

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<212> DNA
<213> Staphylococcus caprae

<220>
<223> CIP 104000 T (ATCC 35538)

<400> 58
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gcaaatttag atagcgtacc ttctgatatt caaacagcag tacgtaacaa tggtggcggt 180

cacttaaacc actcattatt ctgggaatta ttatcaccta attctgaaga aaaaggtgaa 240

gttgtagaca aaatcaaaga acaatggggc tctttagatg aattcaaaaa agaattcgct 300

gacaaagcag cagctcgttt cggttcaggt tgggcttggt tagtagtaaa caacggtcaa 360

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ttaggttta 429

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<212> DNA
<213> Staphylococcus carnosus subsp. Carnosus

<220>
<223> CIP 103274 T (ATCC 51365)

<400> 59
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gctaatttag acagcgtacc atctgacatc caaactgcag ttcgtaataa cggtggtgga 180

catttaaacc attcattatt ctggcaactt ctaacaccta attctgaaga aaaaggtaca 240

gtaattgata aaatcaaaga agaatggggt tctttagaca aatttaaaga tgaatttgct 300

aaaaaagctg ctggacaatt tggttcaggt tgggcatggc tagttgtaga taaagacggt 360

aaactagaaa tcgtttctac tcctaaccaa gacaatccaa tcacagaagg caaaactcct 420

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<210> 60
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<213> Staphylococcus carnosus subsp. Utilis

<220>
<223> CIP 105758 T (DSM 11676)

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gctaatttag acagcgtacc atctgacatc caaactgcag ttcgtaataa cggtggtgga 180

catttaaacc attcattatt ctggcaactt ctaacaccta attctgaaga aaaaggtaca 240

gtaattgata aaatcaaaga agaatggggt tctttagaca aatttaaaga tgaatttgct 300

aaaaaagctg ctggacaatt tggttcaggt tgggcatggc tagttgtaga taaagacggt 360

aaactagaaa tcgtttctac tcctaaccaa gacaatccaa tcacagaagg caaaactcct 420

attttaggac tt 432

<210> 61
<211> 429
<212> DNA
<213> Staphylococcus choromogenes

<220>
<223> CIP 81.59 T (DSM 20454)

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gctaacttaa atagcgtacc agaagataaa caaactcctg tacgtaataa tggtggcggt 180

cacttaaacc actctttatt ctggcaatta ctttcaccac aatcagaaga aaaaggtgaa 240

gtcgtagata aaattaaaga gcaatggggc tctttagatg atttcaaaaa agaatttgca 300

gacaaagcag cagctcgttt tggttctggt tgggcatggc tcgttgtaaa taatggtcaa 360

ttagaaatcg ttactacacc aaaccaagac aacccaattt ctgaaggtaa aactcctatc 420

ttaggatta 429

<210> 62
<211> 429
<212> DNA
<213> Staphylococcus cohnii subsp. Cohnii

<220>
<223> CIP 81.54 T (ATCC 29974)

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gcaaatttag acagtgtacc agaagatatt caaacagctg ttagaaataa tggcggtgga 180

cacttaaacc actcattatt ctgggaatta ttaactccaa actctgaaga aaaaggaact 240

gtagttgata aaattaaaga acaatggggt tctttagatg catttaaaga agaatttgca 300

gataaagctg cagctcgttt tggttcagga tgggcttggc tagttgttaa taatggtaat 360

ttagaaattg ttacaactcc aaaccaagat aacccactta cagaaggtaa aacaccaatc 420

ctaggctta 429

<210> 63
<211> 429
<212> DNA
<213> Staphylococcus cohnii subsp. Urealyticum

<220>
<223> CIP 104024 T (ATCC 49330)

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gcaaatttag acagtgtacc agaaaatatt caaacagctg ttagaaataa tggtggtgga 180

cacttaaacc attcattatt ctgggaatta ttaactccaa actctgaaga aaaaggaact 240

gtagttgata aaattaagga acaatggggt tctttagatg catttaaaga agaatttgca 300

gataaagctg cagctcgttt tggttcaggt tgggcttggc tagttgttaa taatggcaat 360

ttagaaattg ttacaactcc aaaccaagat aacccattaa ctgaaggtaa aacacctatc 420

ttaggctta 429

<210> 64
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<212> DNA
<213> Staphylococcus condimenti

<220>
<223> CIP 105760 T (DSM 11674)

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aaattaaatg cagcaatcga aggtactgat ttagaaaata aatctatcga agaaatcgtt 120

gcaaatttag acagcgtacc atctgacatc caaactgcag ttcgtaataa tggtggtgga 180

catctaaacc attcattatt ctggcaactt ctaacaccta attctgaaga aaaaggtaca 240

gtaattgata aaatcaaaga agaatggggt tctttagaca aattcaaaga tgaatttgct 300

aaaaaagctg ctggacaatt tggttcaggt tgggcttggc tagttgtaga taaaaacggt 360

aacttagaaa tcgtttctac tccaaaccaa gacaacccaa ttacagaagg caaaactcct 420

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<210> 65
<211> 429
<212> DNA
<213> Staphylococcus delphini

<220>
<223> CIP 103732 T

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aaattaaatg ctgctgttga aggtactgaa tttgaaaata aatcattaga agatttaatt 120

gcaaacttag acagcgtacc agaaaactta cgtacagcag ttcgtaataa tggtggcggt 180

cacttaaatc actctatttt ctggcaaatc ttaacaccta actcagaaga aaaaggtgaa 240

gttgtcgata aaattaaaga acaatggggt tctttagatg aattcaaaaa cgaatttgca 300

gacaaagcag ctggccgttt cggttcaggt tgggcttggc ttgttgttaa caacggtaaa 360

ttagaaatcg ttacaactgc aaaccaagat agtccattaa ctgatggttt aacaccaatt 420

ttagcgtta 429

<210> 66
<211> 429
<212> DNA
<213> Staphylococcus epidermidis

<220>
<223> CIP 81.55 T (ATCC 14990)

<400> 66
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aaattaaatt cagcagttga agggacagat ttagaagcta aatcaatcga agaaattgtt 120

gctaatttag atagtgtgcc atctaatatt caaacagctg ttcgtaataa tggcggtggt 180

caccttaacc attcattgtt ctgggaacta ttatcaccaa attctgaaga aaaaggtgaa 240

gtagtagata aaattaaaga acaatggggt tctttagatg aatttaaaaa agaatttgca 300

gataaagctg cagcacgctt tggttcagga tgggcttggt tagttgtaaa caatggacaa 360

ttagaaattg ttacaacacc aaatcaagat aatccaatta ctgaaggaaa aacaccaatt 420

ttaggttta 429

<210> 67
<211> 429
<212> DNA
<213> Staphylococcus equorum

<220>
<223> CIP 103502 T (ATCC 43958)

<400> 67
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gcaaacttag acagtgtacc agaaaacatt caaacagctg ttcgcaataa tggtggagga 180

cacttaaacc attcattatt ctgggaatta ttaactccaa actctgaaga aaaaggtact 240

gttgttgata aaattaaaga acaatggggt tctttagatg cattcaaaga agagtttgct 300

aaccaagctg cagcacgttt cggttcaggt tgggcatggc tagttgtaaa cgatggtaaa 360

ttggaaatcg ttactacacc taatcaagat aacccattaa ctgaaggtaa aacacctatc 420

ctaggctta 429

<210> 68
<211> 429
<212> DNA
<213> Staphylococcus felis

<220>
<223> CIP 103366 T (ATCC 49168)

<400> 68
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aaattaaacg ctacagtaga aggttcagat ttagaaaata aatctcttga agatcttatt 120

gccaatgtag atagtcttcc agaagacaag aaaacagctg tacgtaataa tggtggcggt 180

catcttaacc actcattctt ctgggcactt ttaacaccta attctgaaga aaaaggtgaa 240

gtagttgata aaatcaatga aaaatggggc tcattagacg cattcaaaaa agaatttggc 300

gatgcggctg ctggtcgatt tggttcaggc tgggcatggt tagttgtgaa caatggtgaa 360

ttagaaattg tttcaacacc taaccaagac aatccattgt ctgaaggtaa aacgccaatt 420

ttagctctt 429

<210> 69
<211> 429
<212> DNA
<213> Staphylococcus gallinarum

<220>
<223> CIP 103504 T (ATCC 35539)

<400> 69
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gcaaacttag acagtgtacc agaaaatatt caaacagctg ttagaaataa tggtggtgga 180

cacttaaacc actcattatt ctgggaatta ttaactccta actctgaaga aaaaggtact 240

gtagttgata aaattaaaga acaatggggt tctttagatg catttaaaga agaatttgca 300

gataaagctg cagcacgctt tggttcaggt tgggcatggc tagttgtaaa taacggtaac 360

ttagaaatcg ttactacacc taaccaagac aaccctatta ctgaaggtaa aacacctatc 420

ttaggttta 429

<210> 70
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<212> DNA
<213> Staphylococcus haemolyticus

<220>
<223> CIP 81.56 T (ATCC 29970)

<400> 70
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gctaatttag atagtgtacc tgaagatatt caaacagctg ttcgtaataa tggtggcgga 180

cacttaaatc actcattatt ctgggaatta ttaactccta attctgaaga aaaaggtact 240

gttgttgata aaatcaaaga acaatggggc tctttagatg aattcaaaaa agaattcgct 300

gacaaagcag cagctcgttt cggttcaggt tgggcatggt tagtagttaa caatggtcag 360

ttagaaattg ttactacacc taaccaagat aacccattaa cggaaggtaa aacacctatc 420

ttaggttta 429

<210> 71
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<212> DNA
<213> Staphylococcus hominis subsp. Hominis

<220>
<223> CIP 81.57 T (ATCC 27844)

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gcaaatttag atagtgtatc tgaaaatatt caaacagcag tacgtaataa tggtggaggt 180

catttaaatc actcattatt ctgggaatta ttaactccta attctgaaga aaaaggtact 240

gtagttgata aaattaaaga acaatggggt tctttagatg agtttaaaaa agaattcgct 300

gataaagctg cagcacgttt tggttcaggt tgggcttggt tagtagtaaa taatggaaaa 360

ttagaaattg ttactactcc aaatcaagat aaccctatta ctgaaggaaa aactccaatt 420

ttaggctta 429

<210> 72
<211> 429
<212> DNA
<213> Staphylococcus hominis subsp. Novobiosepticus

<220>
<223> CIP 105719 T (ATCC 700236)

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gcaaatttag atagtgtacc tgaaaatatt caaacagcag tacgtaataa tggtggaggt 180

catttaaatc actcattatt ctgggaatta ttaactccta attctgaaga aaaaggtact 240

gtaattgata aaattaaaga acaatggggt tctttagatg agtttaaaaa agaattcgct 300

gataaagctg cagcacgttt tggttcaggt tgggcttggt tagtagtaaa taatggaaaa 360

ttagaaattg ttactactcc aaatcaagat aaccctatta ctgaaggaaa aactccaatt 420

ttaggctta 429

<210> 73
<211> 429
<212> DNA
<213> Staphylococcus hyicus

<220>
<223> CIP 81.58 T (ATCC 11249)

<400> 73
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aaattaaacg acgctgtaaa aggtacagag ttagaagata aatctattga agagcttatc 120

gcgaatgttg accaattacc tgaggataaa aagactgcgg ttcgtaacaa tggtggcggt 180

cactttaacc attctttatt ctggcaattt ttatccccag aatctgaaga aaaaggtgaa 240

gttgttgaca aaattaaaga acaatggggt tctttagacg catttaaaaa agaattctca 300

gataaagcag cagcacgatt tggatctggc tgggcttggc ttgtagtaaa taatggtcaa 360

ttagaaattg ttacaacagc aaaccaagat agcccattat cagaaggtaa gacaccaata 420

ctcgctcta 429

<210> 74
<211> 429
<212> DNA
<213> Staphylococcus intermedius

<220>
<223> CIP 81.60 T (ATCC 29663)

<400> 74
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aaattaaatg ctgctgttga aggtactgaa tttgaaaata aatcattaga agatttaatt 120

gcaaacttaa atagtgtacc tgaaaacatt cgtacagcgg tacgtaataa tggtggcggt 180

cacttaaatc actctatttt ctggcaactt ttaacaccta actcagaaga aaaaggtgaa 240

gttgtagata aaatcaaaga acaatggggt tctttagatg aatttaaaaa cgaatttgcg 300

gataaagcag cagcacgttt cggttcaggt tgggcttggc ttgttgtcaa taacggcaaa 360

ttagaaatcg ttacaacagc aaaccaagac agtccattaa ctgacggatt atcaccaatc 420

ttagcatta 429

<210> 75
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<212> DNA
<213> Staphylococcus kloosii

<220>
<223> CIP 103503 T (ATCC 43959)

<400> 75
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gcaaacttag acagtgttcc tgaaaacatt caaacagctg ttcgtaataa tggtggggga 180

catattaacc attcattatt ctgggaatta ttaactccta actctgaaga aaaaggtact 240

gtagtagata aaattaaaga acaatggggt tctttagatg catttaaaga agaatttgct 300

gataaagctg caggccgttt cggttcaggt tgggcatggt tagtagtaaa taacggtaac 360

ttagaaatcg ttactacacc taaccaagac aatccattaa ctgaaggtaa aacacctatc 420

ttaggttta 429

<210> 76
<211> 432
<212> DNA
<213> Staphylococcus lentus

<220>
<223> CIP 8163 T (ATCC 29070)

<400> 76
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aaactaaatg atgcagttaa aggtactgac ttagaaagta aatctattga agatattatt 120

aaaaacttaa attctgtacc agatgatatc cgtactgcag ttcaaaacaa tggtggcgga 180

cattacaatc actcattatt ctgggagatg ttaactccaa atgcttctga accatcaggc 240

gaagtagtag atgcaatcag ttctactttc ggttcattag acaaatttaa agaagagttt 300

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gaattatcaa tcgtttcaac tccaaaccaa gataacccat tatctgaagg taaaattcct 420

gtattaggat ta 432

<210> 77
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<212> DNA
<213> Staphylococcus lugdunensis

<220>
<223> CIP 103642 T (ATCC 43809)

<400> 77
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gccaatttag atagcgttcc tgaaaacatt caaacagctg tacgtaataa tggtggtgga 180

cacttaaacc attcactatt ctgggaattt ttaactccta attctgaaga aaaaggtact 240

gtagttgata aaattaaaga acaatggggt tctttagatg aattcaagaa agaattcgct 300

gacaaagctg caggtcgttt tggttcaggt tgggcatggt tagttgtaaa taacggtaaa 360

ttagaaattg ttacaacgcc taaccaagac aacccattaa ctgaaggaaa aacacctatc 420

ttatgtata 429

<210> 78
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<212> DNA
<213> Staphylococcus lutrae

<220>
<223> CIP 105399 T (ATCC 700373)

<400> 78
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aaattaaatg ctgctgttga aggcacagaa ttggaaaata aatcacttga agatttaatc 120

acacatttag atcgcgtacc tgaaaatgta cgtactgctg tgcgtaacaa tggtggcggt 180

catttaaatc actcattttt ctggcaactg cttacaccaa actctgaaga aaaaggtgaa 240

gtagtggata aaattaaaga acaatgggga tcattagacg cattcaaaga agaatttgca 300

gataaagcag cgggtcgttt cggttctggt tgggcttggc ttgttttaaa taatggaaaa 360

ttagaaatta ttacaacacc taaccaagac agtccgttaa ctgaaggttt aacaccgctt 420

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<210> 79
<211> 429
<212> DNA
<213> Staphylococcus muscae

<220>
<223> CIP 103641 T (ATCC 49910)

<400> 79
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gcaaacttaa atgatgtacc tgaagaaaaa cgcacagctg tacgtaataa tggtggcggc 180

cacttaaacc actcattatt ctggcagtta ttaacaccta attcagaaga aaaaggtaca 240

gtggttgaaa aaatcactga aaaatggggt agcttagata gtttcaaaca agaatttgcc 300

gataaagcag cagctcgatt cggttcaggt tgggcatggt tagttgtaga caatggcgag 360

ttagcgattg tgacaactcc aaatcaagac aatccaatca cagatggaaa aactccacta 420

ttaggtctt 429

<210> 80
<211> 429
<212> DNA
<213> Staphylococcus pasteuri

<220>
<223> CIP 103540 T (ATCC 51129)

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aaattaaacg ctgcagttga aggtactgat ttagaagcta aatcaatcga agaaattgta 120

gctaatttag acagtgtacc ttctgatatc caaactgctg ttagaaataa tggtggcgga 180

cacttaaacc actcattatt ctgggaatta ctaacaccta actcagaaga aaaaggtgaa 240

gtagtagata aaattaaaga acaatggggt tctttagatg aattcaaaaa agaatttgct 300

gacaaagcag cagctcgttt tggttcaggt tgggcttggt tagtagtaaa taacgggcaa 360

ttagaaatcg ttacaactcc aaaccaagat aatccattaa ctgaaggtaa aacacctatc 420

ttaggttta 429

<210> 81
<211> 432
<212> DNA
<213> Staphylococcus piscifermentans

<220>
<223> CIP 103958 T (DSM 7373)

<400> 81
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aaattaaatg cagcaatcga aggtactgat ttagaaaata aatcaatcga agaaatcgtt 120

gctaatttag acagcgtacc atcagacatc caaactgcag ttcgtaataa tggtggggga 180

cacttaaacc actcattatt ctggcaactt cttacaccta attctgaaga aaaaggtact 240

gtaattgata aaattaaaga agaatggggc tctttagata aattcaaaga tgaatttgct 300

aaaaaagctg ctggacaatt tggttcaggt tgggcatggc tagttgtaga taaaaacggt 360

aaattagaaa tcgtttctac accaaaccaa gacaacccta ttacagaagg caaaactcct 420

attttaggct ta 432

<210> 82
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<212> DNA
<213> Staphylococcus pulvereri

<220>
<223> CIP 104364 T (DSM 9930)

<400> 82
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aaattaaatg acgcagttaa aggtacagat ttagaaagta aatctattga agatatcatt 120

aaaaatttaa attctgttcc tgaaaatatt cgtactgcag ttcaaaacaa tggtggcgga 180

cattataatc actcattatt ctgggaacta ttaacaccaa atgcttctga accttcagga 240

gaagttgtgg atgcaattag ttctacattc ggttcattag acaaattcaa agaagaattt 300

gcagctgcag cagctggccg tttcggttca ggttgggcat ggttagttgt agataacggt 360

gaattagcga tcgtttcaac tccaaaccaa ggtaacccta tttcagaagg taaacttcca 420

gtattaggct ta 432

<210> 83
<211> 429
<212> DNA
<213> Staphylococcus saccharolyticus

<220>
<223> CIP 103275 T (ATCC 14953)

<400> 83
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aaattaaact cagcagttga aggaacagat ttagaagcta aatcaatcga agaaattgtt 120

gctaatttag acaatgtccc atcaaatatt caaacagctg ttcgtaacaa tggcggtgga 180

catttaaacc attcattatt ctgggaatta ttatcaccta actcagaaga aaaaggtgaa 240

gttgtagata aaattaaaga acaatggggt tctttagatg aatttaaaaa agaatttgcg 300

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gttgttgata aaattaaaga acaatggggc tctttagatg catttaaaga agaatttgct 300

gacaaagcag cagctcgttt cggttcaggt tgggcatggc tagttgtgaa taacggtaac 360

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gcaaacttag acagtgttcc agaaaatatt caaacagctg ttcgaaataa tggtggtgga 180

cacttaaacc actcactatt ctgggaatta ttaactccaa actcagaaga aaaaggtact 240

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gacaaagcag cagctcgttt cggttcaggt tgggcatggc tagttgtgaa taacggtaac 360

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cacttaaacc actcattatt ctggcaaatc ctttcaccta actctgaaga gaaaggtaca 240

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atcctaggct ta 432

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<223> CIP 104850 T (ATCC 51145)

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gacaaagctg cagctcgttt tggttcaggt tgggcttggt tagttgttaa taatggtcaa 360
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catttaaacc actcattatt ctgggaatta ttaactccaa actcagaaga aaaaggtact 240
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<220>
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<220>
<223> D1

<400> 95
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<220>
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<220>
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<400> 96
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(74) Agents: **MARTIN, Jean-Jacques** et al.; Cabinet Regimbeau, 20, rue de Chazelles, F-75847 Paris Cedex 17 (FR).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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**Published:**
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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 01/088186 A3

(54) **Title:** METHODS FOR DETECTING AND IDENTIFYING A GRAM POSITIVE BACTERIA IN A SAMPLE

(57) **Abstract:** The present invention provides fragments of a sodA gene from gram positive bacteria, methods of using these fragments as probes to detect and identify microorganisms in a sample and kits containing suitable reagents to perform the method.

# INTERNATIONAL SEARCH REPORT

ational Application No
../IB 01/01155

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EPO-Internal, MEDLINE, PAJ, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | POYART CLAIRE ET AL: "Sequencing the gene encoding manganese-dependent superoxide dismutase for rapid species identification of enterococci." JOURNAL OF CLINICAL MICROBIOLOGY, vol. 38, no. 1, January 2000 (2000-01), pages 415-418, XP002205503 ISSN: 0095-1137 | 1-5,7, 14-16,20 |
| Y | the whole document | 6,12,13, 18 |
| | & DATABASE GENBANK 'Online! NCBI; AJ387941, 7 January 2000 (2000-01-07) POYART, C. ET AL.: "Enterococcus avium partial sodA gene for superoxide dismutase, strain NEM1630" the whole document | |
| | --- | |
| | -/-- | |

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 7 October 2002 | 21. 10. 2002 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | Botz, J |

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | POYART CLAIRE ET AL: "Identification of streptococci to species level by sequencing the gene encoding the manganese-dependent superoxide dismutase." JOURNAL OF CLINICAL MICROBIOLOGY, vol. 36, no. 1, January 1998 (1998-01), pages 41-47, XP002205504 ISSN: 0095-1137 | 1-3,14, 15 |
| Y | the whole document | 4-6,12, 16,18 |
| | --- | |
| Y | KAWAMURA YOSHIAKI ET AL: "Genetic approaches to the identification of the mitis group within the genus Streptococcus." MICROBIOLOGY (READING), vol. 145, no. 9, 1999, pages 2605-2613, XP002205505 ISSN: 1350-0872 figures 1-3; tables 1,2 | 1-5,7, 13-16,20 |
| | --- | |
| Y | GAILLOT O ET AL: "Molecular characterization and expression analysis of the superoxide dismutase gene from Streptococcus agalactiae" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, ELSEVIER SCIENCE PUBLISHERS, BARKING, GB, vol. 204, no. 1-2, 19 December 1997 (1997-12-19), pages 213-218, XP004100715 ISSN: 0378-1119 the whole document | 1-5,7, 13-16,20 |
| | --- | |
| X | POYART C ET AL: "Characterization of superoxide dismutase genes from gram-positive bacteria by polymerase chain reaction using degenerate primers." FEMS MICROBIOLOGY LETTERS. NETHERLANDS 15 AUG 1995, vol. 131, no. 1, 15 August 1995 (1995-08-15), pages 41-45, XP002215739 ISSN: 0378-1097 | 1-3 |
| Y | paragraphs ‘02.2!,‘02.3!,‘03.1! | 5,6,12, 14-16,18 |
| | --- | |
| | -/-- | |

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | DATABASE EBI 'Online!<br>EMBL; 11 March 1999 (1999-03-11)<br>CLEMENTS, M.O. ET AL.: "Staphylococcus aureus..."<br>retrieved from EBI, accession no. AF121672<br>Database accession no. AF121672<br>XP002215740 | 1-3,12 |
| Y | page 3898 -page 3899<br>& CLEMENTS ET AL.: "Characterization of the major Superoxide Dismutase of Staphylococcus aureus and its role in starvation survival, stress resistance, and pathogenicity"<br>JOURNAL OF BACTERIOLOGY,<br>vol. 181, no. 13, July 1999 (1999-07),<br>pages 3898-3903,<br>the whole document<br>--- | 5,6,<br>13-16,18 |
| Y | DATABASE EBI 'Online!<br>EMBL; 16 March 1999 (1999-03-16)<br>BARASH, SC ET AL.: "Staphylococcus aureus contig. SEQ. ID. #426"<br>retrieved from EBI, accession no. AAV74737<br>Database accession no. AAV74737<br>XP002215741 | 1-3,12 |
| Y | the whole document<br>& CA 2 194 411 A (HUMAN GENOME SCI INC.)<br>6 July 1997 (1997-07-06)<br>claims 15,16<br>--- | 5,6,<br>13-16,18 |
| A | SANDERS J W ET AL: "Stress response in Lactococcus lactis: cloning, expression analysis, and mutation of the lactococcal superoxide dismutase gene."<br>JOURNAL OF BACTERIOLOGY. UNITED STATES SEP 1995,<br>vol. 177, no. 18,<br>September 1995 (1995-09), pages 5254-5260,<br>XP002205506<br>ISSN: 0021-9193<br>the whole document<br>----- | 1-7,<br>12-16,<br>18,20 |

# INTERNATIONAL SEARCH REPORT

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

1-7,12-16,18,20 (partially)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-5,7,13-16,20 (partially)

   Invention 1:

   A polynucleotide-probe, in particular the one specified by Sequence Identity Number 1, specific for the sodA-gene of gram-positive bacteria, its use in a method for accurate identification of the species of gram-positive bacteria, in particular from the genus Enterococcus, in a sample, a DNA-chip comprising at least one polynucleotide specified by Sequence Identity Number 1 or a fragment thereof, a kit for the detection of a gram positive bacteria present in a sample containing at least Sequence Identity Number 1.

2. Claims: 1-5,7,8,13-16,20 (partially)

   Inventions 2 - 35:

   Idem for invention 2 to invention 35, inventions being specified by the Sequence Identities derived for the bacterial genus Enterococcus, namely Sequence Identity Number 2-19 and 21-36.

3. Claims: 1,2,5,13,14,16 (partially), 9 (completely)

   Invention 36:

   A polynucleotide-probe, in particular the one specified by Sequence Identity Number 20, specific for the sodA-gene of gram-positive bacteria, its use in a method for accurate identification of the species of gram-positive bacteria, in particular from the species Lactococcus garvieae, in a sample, a DNA-chip comprising at least one polynucleotide specified by Sequence Identity Number 20 or a fragment thereof, a kit for the detection of a gram positive bacteria present in a sample containing at least Sequence Identity Number 20.

4. Claims: 1-3,5,10,13-16,19 (partially)

   Invention 37:

   A polynucleotide-probe, in particular the one specified by Sequence Identity Number 37, specific for the sodA-gene of gram-positive bacteria, its use in a method for accurate identification of the species of gram-positive bacteria, in particular from the genus Streptococcus, in a sample, a DNA-chip comprising at least one polynucleotide specified by Sequence Identity Number 37 or a fragment thereof, a kit for

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

the detection of a gram positive bacteria present in a sample containing at least Sequence Identity Number 37.

5. Claims: 1-3,5,10,13-16,19 (partially)

Inventions 38 - 50:

Idem for invention 38 to invention 50, inventions being specified by the Sequence Identities derived for the bacterial genus Streptococcus, namely Sequence Identity Number 38 - 50.

6. Claims: 1-3,5,11,13-16,21 (partially)

Invention 51:

A polynucleotide-probe, in particular the one specified by Sequence Identity Number 51, specific for the sodA-gene of gram-positive bacteria, its use in a method for accurate identification of the species of gram-positive bacteria, in particular from the genus Abiotrophia, in a sample, a DNA-chip comprising at least one polynucleotide specified by Sequence Identity Number 51 or a fragment thereof, a kit for the detection of a gram positive bacteria present in a sample containing at least Sequence Identity Number 51.

7. Claims: 1-3,5,11,13-16,21 (partially)

Invention 52 - 53:

Idem for invention 52 to invention 53, inventions being specified by the Sequence Identities derived for the bacterial genus Abiotrophia, namely Sequence Identity Number 52 - 53.

8. Claims: 1-3,5,6,12-16,18 (partially)

Invention 54:

A polynucleotide-probe, in particular the one specified by Sequence Identity Number 54, specific for the sodA-gene of gram-positive bacteria, its use in a method for accurate identification of the species of gram-positive bacteria, in particular from the genus Staphylococcus, in a sample, a DNA-chip comprising at least one polynucleotide specified by Sequence Identity Number 54 or a fragment thereof, a kit for the detection of a gram positive bacteria present in a sample containing at least Sequence Identity Number 54.

9. Claims: 1-3,5,6,12-16,18 (partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Invention 55 - 93:

Idem for invention 55 to invention 93, inventions being specified by the Sequence Identities derived for the bacterial genus Staphylococcus, namely Sequence Identity Number 55 - 93.

10. Claims: 1-6,14-16,18-21 (partially)

Invention 94:

A polynucleotide-probe, in particular the one specified by Sequence Identity Number 94, specific for the sodA-gene of gram-positive bacteria, its use in a method for accurate identification of the species of gram-positive bacteria in a sample, a kit for the detection of a gram positive bacteria present in a sample containing at least Sequence Identity Number 94.

11. Claim : 17 (completely)

Invention 95:

A polynucleotide-probe, in particular the one specified by Sequence Identity Number 95 and 96, specific for the sod-gene of gram-positive bacteria, their use in a method for accurate identification of the species of gram-positive bacteria in a sample.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

ational Application No
..../IB 01/01155

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| CA 2194411 A | 06-07-1997 | CA 2194411 A1 | 06-07-1997 |
| | | EP 0786519 A2 | 30-07-1997 |
| | | JP 9322781 A | 16-12-1997 |
| | | US 2002103338 A1 | 01-08-2002 |

Form PCT/ISA/210 (patent family annex) (July 1992)

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